USE OF BIOCHAR FOR REDUCING FEMALE SEX HORMONAL POLLUTION FROM AGRICULTURAL AREAS

by

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ABSTRACT

Poultry and liquid swine manure are major sources of bioactive levels of natural steroidal sex hormones, including 17β-estradiol, estrone, testosterone and progesterone. Although several detailed studies have investigated the environmental pathways and ecotoxicology of high-toxicity-at-low-concentrations of sex hormones in soil and water media, there is a lack of knowledge with respect to the fate and transport of steroid hormones in the soil matrix and aquatic media. Three separate studies were performed with the objective of developing effective on-field remediation techniques in order to reduce the environmental and biological hormonal pollution from agricultural sources. In the first study, the sorption retention and desorption resistance of two types of biochars (fast and slow pyrolysis) for three female sex hormones (17β-estradiol, estrone, progesterone) was evaluated in batch equilibrium experiments. Slow-pyrolysis biochar demonstrated a stronger remediation capability than the fast-pyrolysis biochar in this study. In the second study, the retention potency of slow pyrolysis biochar was assessed as a topsoil amendment on the fate and transport of the three sex hormones in a sandy soil over a 45-day period where poultry and liquid swine manure were applied as fertilizer to outdoor lysimeters, irrigated with different levels of simulated rainfall. A significant difference (p<0.05) was observed between the spatial-temporal stratification of three hormones in lysimeters with soil and soil-biochar treatments. The significant lower mass of hormones quantified at different depths of the soil profile and the measured concentration of all three hormones in collected leachate samples over time in the lysimeters amended with 1% slow pyrolysis biochar, potentially confirmed the hypothesis of this study, confirmed the highly effective retention capability of biochar as a soil-amendment for reducing manure-borne hormonal pollution in soil and water.
RÉSUMÉ

Les lisiers de volaille et de porc sont les principales sources, à des niveaux bioactifs, d'hormones sexuelles stéroïdiennes naturelles tel le 17β-estradiol, l'estrone, la testostérone et la progestérone. Quoique plusieurs études détaillées aient enquêté sur les chaînes de pénétration dans l'environnement et l'écotoxicologie de haute-toxicité-à-faible-concentration des hormones sexuelles dans la matrice du sol et dans les milieux aquatiques, il demeure des lacunes en ce qui concerne le devenir et le transport des hormones stéroïdes dans ces milieux. Trois études distinctes furent réalisées ayant pour objectif de développer des techniques d'assainissement sur le terrain qui permettraient de réduire la pollution hormonale liée aux sources agricoles et ses effets néfastes sur l'environnement et les biotes.

Dans la première expérience, la rétention par sorption et la résistance à la désorption de trois hormones sexuelles féminines (17β-estradiol, estrone, progestérone) en présence de deux types de biochars (pyrolyse lente ou rapide) fut évalué par lots d'échantillons sous conditions d'équilibre. Le biochar produit par pyrolyse lente démontra une plus grande capacité d'assainissement que celui produit par pyrolyse rapide. Dans une seconde étude, entreprise à l'extérieur en lysimètres, le potentiel de rétention d'un biochar produit par pyrolyse lente et servant comme amendement à la couche arable d'un sol sablonneux, fut évalué, sur une période de 45 jours, quant à son effet sur le sort et le transport des trois hormones sexuelles féminines. Chacun des lysimètres reçurent du lisier de volaille ou de porc comme engrais, et fut irrigué avec un niveau particulier de précipitation simulée. Entre le sols ayant ou n’ayant pas reçu d’addition de biochar, une différence significative (p < 0.05) dans la stratification spatio-temporelle des trois hormones dans le sol fut évidente. Pour la durée de l’expérience, les teneurs des trois hormones à différentes profondeurs du profil du sol et dans le lixiviat furent sensiblement moins élevés dans les lysimètres dont le sol avait reçu un amendement de biochar à pyrolyse lente (à 1%), que ceux n’ayant pas reçu d’amendement. Ces résultats confirmèrent l’hypothèse de l’étude qui voudrait que le biochar utilisé comme amendement au sol confère une excellente capacité de rétention, dans le sol et dans l’eau, des polluants hormonaux provenant de lisiers.
CONTRIBUTIONS OF THE AUTHORS

The authorship for the four manuscripts, making up this thesis, is as follows:


(ii) S.Alizadeh, S.O.Prasher. Effect of Slow Pyrolysis Biochar on the Fate and Transport of Manure-Borne Progesterone in Soil (under preparation)

(iii) S.Alizadeh, F.Gobbi, S.O.Prasher. Evaluation of Slow Pyrolysis Biochar in Reducing Estrogen-Based Female Sex Hormones in Poultry Manure (under preparation)

(iv) S.Alizadeh, F.Gobbi, S.O.Prasher. Role of Slow-Pyrolysis Biochar Amendment on the Fate and Transport of Estrogenic Hormones in Liquid Swine Manure (under preparation)

(v) S.Alizadeh, F.Gobbi, S.O.Prasher. Influence of Biodegradation and Chemical Transformation and Photo-degradation on the Fate and Transport of Female Sex Hormones in the Presence of Slow Pyrolysis of Biochar (under preparation)

All authors are from the Department of Bioresource Engineering, Macdonald Campus, McGill University, Montreal. All the fieldwork, analysis of data, and preparation of above manuscripts were completed by the candidate, S.Alizadeh and F.Gobbi under the supervision of Dr. Shiv O. Prasher, James McGill Professor, and Department of Bioresource Engineering. Mr.Gobbi was a visiting student from Italy. Dr. Prasher provided the necessary funds, supervisory guidance, and assistance to carry out this research.
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# LIST OF SYMBOLS AND ABBREVIATIONS

<table>
<thead>
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<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeter (100 cm = 1 meter)</td>
</tr>
<tr>
<td>cmol</td>
<td>Centimole, unit for CEC (cation exchange capacity)</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
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<tr>
<td>GC</td>
<td>Gas Chromatography</td>
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<tr>
<td>ha</td>
<td>Hectare</td>
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<tr>
<td>HPLC</td>
<td>High Performance Chromatography</td>
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<tr>
<td>i.e.</td>
<td>That is</td>
</tr>
<tr>
<td>K&lt;sub&gt;d&lt;/sub&gt;</td>
<td>Soil-water partitioning coefficient</td>
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<tr>
<td>kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>K&lt;sub&gt;oe&lt;/sub&gt;</td>
<td>Adsorption coefficient</td>
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<tr>
<td>K&lt;sub&gt;sat&lt;/sub&gt;</td>
<td>Saturated hydraulic conductivity</td>
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<tr>
<td>L</td>
<td>Liter</td>
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<tr>
<td>Mg</td>
<td>Milligram</td>
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<tr>
<td>min</td>
<td>Minute</td>
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<tr>
<td>mL</td>
<td>Milliliter</td>
</tr>
<tr>
<td>O.S.</td>
<td>Organic solvent</td>
</tr>
<tr>
<td>Pr</td>
<td>Statistical probability</td>
</tr>
<tr>
<td>r</td>
<td>Correlation coefficient</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolution per minute</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>μL</td>
<td>Microliter</td>
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<tr>
<td>μg</td>
<td>Microgram</td>
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Chapter 1
General Introduction

1.1 Problem Statement

Over the last decade, expanding anthropogenic activities, including modern animal feeding operations, have generated excessive quantities of organic wastes (i.e., animal manure and biosolids). The land application of these nutrient-rich (N, P, K) organic wastes as fertilizer constitutes a multi-purpose and economically viable waste-management strategy. Every year $3.4 \times 10^6$ ha of Canadian land receive animal manure as an amendment to improve soil fertility and tilth, and thereby enhance crop growth. However, this waste management technique has been identified as one of the major gateways for the environment’s exposure to new classes of toxic and high-risk organic contaminants known as emerging contaminants. Having endocrine-disrupting properties, some at concentrations as low as ng L$^{-1}$, these contaminants represent a high chronic toxicity risk and are associated with adverse long-term health issues (e.g. carcinogenicity, mutagenicity or teratogenicity (Choi et al., 2004). Recent environmental studies have therefore focused on the prevalence, fate, transport pathways and ecotoxicology of these previously sparsely-documented manure-borne biologically-active contaminants.

Amongst the emerging contaminants of greatest concern are the natural steroidal sex hormones (i.e., $17\beta$-estradiol (E2), estrone (E1), testosterone, and progesterone) due to their adverse developmental and carcinogenetic effects. The advancement of analytical chemistry approaches and chromatography techniques have provided the means to detect, identify and quantify of steroid sex hormones in aquatic resources, soils matrices and aquatic biota. Several incidences of developmental and reproductive abnormalities in aquatic environments and a significant incidence of female endocrine disorders such as polycystic ovary syndrome (PCOS) and breast cancer, along with prostate and testicular cancers in men (Pruden et al., 2006) have confirmed the risks of manure-borne hormonal pollution in aquatic environments.
At least 90% of the total load of female sex hormones passed on to the environment is estimated to arise from the application of livestock manure (Maier et al., 2009). The environmental behavior of sex hormones is influenced by several mechanisms and parameters. Among the predominant factors determining the fate and transport of manure-borne steroidal hormones in the environment are chemical and physical processes such as sorption to soil and sediment, and both biotic and abiotic transformation/degradation rates (Lai et al., 2002). However, recent studies focusing on these contaminants’ presence in soil and water suggest that knowledge of new remediation techniques to reduce environmental hormonal pollution remains insufficiently detailed.

1.1.1 A novel approach for reducing aquatic hormonal pollution

The strong sorption affinity for poly-aromatic hydrocarbons (PAHs) and other categories of organic contaminants to carbon-rich amendments (e.g., black carbon, activated carbons and biochar) may render possible their use as a novel multi-purpose and financially viable approach to reducing the bioavailability and bioaccumulation of organic contaminants. Biochar is the by-product of the thermo-chemical decomposition of biomass and biological residues in the absence of oxygen. Due to its resistance to biodecomposition, high specific surface area, nano-scale condensed aromatic rings, micro-scale crystalline structure and macro-scale amorphous structure, biochar offers a strong sorption affinity for inorganic contaminants (e.g., heavy metals) and hydrophobic organic contaminants. Biochar can be applied directly to the soil and provide a convenient way of disposing of organic waste; it provides the added value of binding pollutants, reducing their bioavailability, while promoting plant growth and stimulating ecological restoration.

1.2 Objectives

The main goal of this research project was to evaluate the impact of biochar’s retention ability on three manure-borne female sex hormones (17β-estradiol, estrone and progesterone) levels in an agricultural soil. Batch equilibrium studies served in making an initial comparative assessment of the sorption ability and desorption resistance of the two biochar types. To the best of our knowledge, this study is the first to report the sorption-
desorption behavior of progesterone in biochar-amended soil, along with the desorption isotherms of these three female sex hormones in the same medium.

In order to validate the proposed remediation ability of biochar under a real case scenario, an outdoor-lysimeter study was conducted with the following objectives:

1. To investigate the fate and transport of three manure-borne steroidal sex hormones (E2 and E1 and progesterone) in a sandy soil amended with slow pyrolysis biochar.
2. To evaluate the potential of slow-pyrolysis biochar topsoil amendments in reducing hormonal pollution from poultry and swine manure application.
3. To investigate the influence of biochar on photo-degradation, microbial degradation and chemical transformation of the three aforementioned female sex hormones

1.3 Thesis organization

This thesis is divided into seven chapters. Chapter 1 briefly introduces the new class of emerging contaminants: steroid sex hormones. It details their environmental risks and long-term adverse health effects, along with a novel approach proposed for reducing aquatic pollution caused by these hormones. This chapter also presents the objectives of the study.

Chapter 2 reviews the literature regarding emerging contaminants and their environmental occurrence and pathways, with an emphasis on steroid sex hormones. It presents their physico-chemical properties, summarizing their environmental fate and transport, together with their potential impacts. Chapter 2 also reviews the environmental behavior of steroid sex hormones including their sorption/desorption mechanisms in soil media and biodegradation and abiotic transformation. The last section of the literature review is focused on the background of biochar, its structural properties and environmental remediation potential as a soil amendment.

Chapter 3 presents a detailed comparative assessment of the sorption ability and desorption resistance of slow vs. fast pyrolysis biochar for three hormones in sandy soil. The sorption and desorption isotherms of each of three female sex hormones were
developed. To the best our knowledge, this was the first investigation to evaluate the sorption-desorption behavior of progesterone and to report on the desorption isotherms of female sex hormones in biochar-amended soil.

**Chapter 4, 5 and 6** presents the methodology and results of the lysimeter study for three hormones. The focus of these three chapters is on the influence of slow-pyrolysis biochar amendment on the fate and transport of manure-borne estrogens and progesterone. The spatiotemporal stratification of each hormone in the soil profile and water samples, the statistical analysis and mass balances are provided in each chapter.

**Chapter 7** presents the methodology and the results of assessment of the influence of biotic (microbial) and abiotic (chemical and photo-degradation) transformation of the three female sex hormones, 17β-estradiol (E2), estrone (E1) and progesterone (P) and the effect of slow pyrolysis biochar amendment on these mechanisms.

Finally, **Chapter 8** concludes this thesis by summarizing the entire work of evaluating the remediation potential of biochar for reducing aquatic hormonal pollution and the field-scale investigation of the fate and transport of three manure-borne female sex hormones. **Chapter 8** also summarizes the major findings of this study and gives recommendations for future work.
Chapter 2
General Review of Literature

2.1 Introduction

2.1.1 Emerging contaminants

Over the last decade, the development of advanced analytical chemistry approaches and chromatography techniques [e.g., high performance liquid chromatography (HPLC) and gas chromatography equipped with mass spectrometry (GC-MS) and (GC-MS/MS)], due to their detection sensitivity and accuracy, has provided the means to detect, identify and quantify extremely–low concentrations of newly identified hazardous contaminants in aquatic resources (i.e., drinking water, surface and groundwater, wastewater, soils matrix water) and such biological chains as aquatic biota (Mastroianni et al., 2011). Known as emerging contaminants, these biologically-active, toxic organic micro pollutants are poorly documented in terms of both the toxicological and biological risks they pose to the environmental, and the attendant health and safety issues (Schriks et al., 2010). The interaction between these compounds and their receptors causes reactions which can eventually lead to adverse ecological or long-term human health effects. The latter can include serious human pandemics, cancer, adverse pregnancy outcomes and hormonally-related abnormalities of reproduction and development.

A sustained exposure to low concentrations of these compounds can induce effects that may only be visible over generations (Daughton and Ternes, 1999). The broad spectrums of emerging contaminants include anthropogenic chemicals synthesized as a result of expanding human activities. The environment is predominantly exposed to these through natural physicochemical or biological processes (Daughton, 2004). Pharmaceuticals, personal-care products (PCPs), steroids and hormones, surfactants, perfluorinated compounds (PFCs), flame retardants, industrial additives and agents, and gasoline additives, as well as their transformation products and nano-materials have been classified as paramount emerging contaminants (Farré et al., 2008).
2.1.2 Environmental occurrence and pathway

Expanding anthropogenic and agricultural activities represent the major uncontrolled and non-point environmental sources of these contaminants. These sources originate in massive production of industrial chemicals and pharmaceuticals, the implementation of Concentrated Animal Feeding Operations (CAFOs), inappropriate wastewater disposal, as well as through land development management practices which purposefully introduce potential contaminants (i.e., pesticide application, groundwater recharge, sewage sludge application) into the environment (Daughton, 2004). Combining endocrine-system-disrupting properties with the ability to induce severe chronic toxicity and adverse, long-term health issues (e.g. carcinogenicity, mutagenicity or teratogenicity) at concentrations as low as ng L⁻¹ (Choi et al., 2004), most of these organic pollutants have been characterized as Endocrine Disrupting Compounds (EDCs).

2.1.3 Endocrine disrupting compounds

The endocrine system is an intricate, physiological regulatory system with a fundamental role in major metabolic processes including reproductive, growth, cardiovascular and neurological functions (Damstra et al., 2002). The main role of the endocrine system is the production of hormones, which, by traveling in the blood stream and binding to special receptors in target organs and tissues, function as extremely–low-concentration chemical signals. Furthermore, the endocrine system affects secondary hormone secretion, cell differentiation and gene expression. The paramount role of the endocrine system is to protect the body from the detrimental effects of fluctuations in hormone levels/responses and to maintain a hormonal balance (i.e. homeostasis) (Fang et al., 2000).

Including a variety of synthetic and industrial compounds and pesticides such as 1,1,1-trichloro-2,2-bis(p-chlorophenyl)-ethane (DDT), dioxins, polychlorinated biphenyls (PCBs) and nonylphenols (Gould et al., 1998), EDCs are exogenous substances which modulate the functions of the endocrine system by mimicking, counteracting, altering or interfering with the metabolism and biosynthesis of endogenous hormones (Colucci et al., 2001). Among EDCs, natural and synthetic steroidal sex hormones have attracted the
attention of scientists due to their carcinogenic and other ill effects. These compounds influence physiological functions by interfering with the normal endocrine system, which results in sexual and reproductive abnormalities and intersexuality in the aquatic environment. The potential high-risk steroid sex hormones are 17β-estradiol and its primary metabolites, estrone, progesterone, testosterone and cortisol.

2.2 Steroid Sex Hormones

2.2.1 Physical and structural properties

Steroid sex hormones are cholesterol-derived biochemicals consisting of a common carbon skeleton comprising of four fused ring (a cyclopentan-α-perhydrophenanthrene ring). The number and location of the double bonds, along with the structural and stereochemical characteristics of the functional groups along the carbon skeleton result in different physiochemical properties (Ying et al., 2002). Moderately hydrophobic (i.e., slightly soluble in water), these weak acids of low volatility are classified in three major categories: Estrogens and progestagens (female hormones), and androgens (male hormones; (Bevacqua et al., 2011). The primary estrogens are 17β-estradiol (E2) and its metabolite estrone (E1). Their structural difference is due to variations in the C-16 and C-17 positions of the D-ring. Estradiol has a hydroxyl group in the C-17 position which can be in either an α or β conformation, whereas estrone bears a carbonyl group at the C-17 position (Bevacqua et al., 2011).

Fig 2.1 Basic steroid hormone structure (27-carbon cholestane). The steroid skeleton is characterized by four fused rings, labeled from A to D. Each carbon is labeled from 1 to 27 (Young and Borch, 2009)
Known as dominant female sex hormones, the estrogens are primarily responsible for the development of female traits, ovulation, reproduction, mating and breeding behavior, and somatic cell function (Jobling et al., 2003). The primary male physiological development including male traits, reproduction, mating and breeding behavior, and secondary sex differentiation are the function of androgens (Fang et al., 2003). Known as early precursors in the formation of other steroid hormones, progesterones are produced by both sexes, with significantly higher concentrations being present in females. In its direct reproductive role, progesterone is responsible for preparing the uterus for the fertilized egg and for maintaining pregnancy. It also plays an important role in the nervous system, influencing mating and parental care behavior (Bevacqua et al., 2011). Given the detection of female sex hormones at concentration exceeding their lowest observed effect level (LOEL) of 10 ng L\(^{-1}\) (Shore and Shemesh, 2003), and these compounds’ potential to induce physiological disorders at lower concentrations than other steroid hormones, they have become the major concern among all EDCs. Both the free and conjugated forms of female hormones are environmentally stable and show a high biotransformation potential (Shore and Shemesh, 2003).

### 2.2.2 Potential impacts of environmental exposure to steroid sex hormones

Extensive evidence of endocrine disruption has been reported (Balabanič et al., 2011; Söffker and Tyler, 2012). The primary adverse health effect of these toxic hormones in aquatic environments include aquatic intersexuality (e.g. feminization and musculation of fish), sex ratio alteration, abnormal blood hormone levels and reproductive and developmental abnormalities. These symptoms represent the most widely reported adverse effects of endocrine disruption arising from the availability of steroid sex hormones in the environment (Young and Borch, 2009). The recently expanding number of diagnoses of female endocrine disorders such as polycystic ovary syndrome (PCOS) and breast cancer, along with numerous cases of prostate and testicular cancers have been linked to the impact of these hormones on humans.

Vitellogenin, a high concentration precursor of an egg-yolk protein in the plasma of sexually mature aquatic biota, is considered as a biological indicator of environmental endocrine disruption and feminization of male fish exposed to natural and synthetic
hormones (Beresford et al., 2011). Li et al. (2006), reported a significant increased level of vitellogenin in male bullfrogs (*Rana catesbeiana* Shaw, 1802) after four weeks’ exposure to E2. This would suggest that there is likely an adverse impact of estrogens on the reproduction in male bullfrogs. A detailed study of endocrine disruption in an aquatic environment was conducted by Guillette and Gunderson (2001). Investigating changes in the reproductive and endocrine systems of juvenile alligators (*Alligator mississippiensis* Daudin, 1802) obtained from contaminated or control lakes in central Florida, they found a significant difference between the in vitro syntheses of E2 in the ovaries of alligators hatched in contaminated vs. control lakes. Lange et al. (2011) demonstrated that exposure to EDCs in wastewater treatment effluent discharges led to a higher percentage of feminization of roach fish (*Rutilus rutilus* Linnaeus, 1758) living in an UK river.

Several other cases of aquatic biological disruption cause by steroid hormones have been documented in a large number of environmental studies. The feminization of male fish or the masculinization of female fishes (Vajda et al., 2011), the alteration of the reproductive biology of wild fathead minnows (*Pimephales promelas* Rafinesque, 1820) and rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792) at aquatic estradiol and estrone concentrations as low as 1-10 ng L\(^{-1}\) and 25-50 ng L\(^{-1}\), respectively, are good evidence of hormonal environmental contamination (Young and Borch, 2009). Blazer et al. (2007) reported intersexuality in the fish exposed to steroid hormones downstream from a wastewater treatment plant and drainage effluents, while Irwin et al. (2001) reported elevated levels of vitellogenin in painted turtles (*Chrysemys picta* Schneider, 1783). Partridge et al. (2010) point out that endocrine chemical disruptors have the potential to alter not only the primary sexual traits and reproduction functions but also secondary sexual traits, thus potentially affecting mating dynamics. They showed that after only 10 days of exposure to a low concentration of the synthetic estrogen 17\(\alpha\)-ethinylestradiol [19-nor-17\(\alpha\)-pregna-1,3,5(10)-trien-20-yn-3,17-diol] adult male pipefish developed female-like secondary sexual traits and a resultant malfunction in mating dynamics with possible long term consequences.
2.3 Steroid Hormone Occurrence and Pathways in the Environment

Continuous excretion from humans (e.g., urine and faeces) and vertebrate livestock (e.g., animal manure and wastewater), along with the output of septic systems and, expanding concentrated animal feeding operations (CAFOs) represent the main sources of steroid sex hormones in the environment. Effluents and sludge from wastewater treatment plants (WWTPs), along with untreated wastewaters are major pathway of environmental exposure to steroids from anthropogenic sources (Liu et al., 2012). Up to 48 ng L\(^{-1}\) and 64 ng L\(^{-1}\) of E2 and E1 have been measured in the effluents from wastewater treatment plants in Canada (Ternes et al. (1999). While considered a practical waste management strategy, surface application of WWTP-generated organic waste to agricultural land has been identified as another dominant source of these hormones in the environment. Consequently the potential exists for hormonal contamination of soil and surface water as well as that of groundwater through infiltration or runoff. From an environmental and health safety perspective, these organic wastes are major sources of bioactive levels of natural steroidal sex hormones, including E2, E1, testosterone and progesterone. The total estimated mass of steroids contributed to the environment by Chinese swine farms is reported to be 139 Mg yr\(^{-1}\) (Liu et al., 2012). The significant estrogens loads contributed to the environment through the traditional spreading of stored pig manure is reported by Combalbert et al. (2012).

The species, sex, age and reproductive status of animals, along with their diet and veterinary treatments were found to effect the levels of hormones they excreted (Lorenzen et al., 2004). For instance, over 90% of the total estrogens excreted by cattle (Bos taurus Linnaeus, 1758) bore the free and conjugated forms of 17α-estradiol, 17β-estradiol and estrone, along with their metabolites. The 17α-estradiol form was more prevalent than 17β-estradiol. Comparatively, in the excreta of swine (Sus scrofa domestica Erxleben, 1777) or poultry (Gallus gallus domesticus Linnaeus, 1758) the free form of 17β-estradiol, along with estrone and estriol plus conjugates are more common and 17α-estradiol is rarely quantified (Hansen et al., 2011). Different animals excrete steroid hormones in different ways. More than 58% of estrogens excreted by cattle are found in their feces, whereas swine and poultry excrete estrogens mostly in their urine.
(96% and 69%, respectively). Shore and Shemesh (2003) reported the hormone (E2) content of different dairy cow (0.24±0.03 mg kg\(^{-1}\)), swine (1.225±0.28 mg kg\(^{-1}\)) and poultry (33±12 μg kg\(^{-1}\)) manures. Finlay-Moore et al. (2000) investigating the hormonal contamination of soil as result of land application of broiler litter to grasslands found mean E2 post-application levels of 0.675 g kg\(^{-1}\) in soils and 2.53 μg L\(^{-1}\) in runoff, compared to 55 ng kg\(^{-1}\) and 50 to 150 ng L\(^{-1}\), respectively, for pre-application background levels. In swine lagoons from farrowing sow operations the concentration of E2 ranged from 2.2 to 3.0 μg L\(^{-1}\) and those of E1 from 9.6 to 24.9 μg L\(^{-1}\) (Hutchins et al., 2007). In a survey of hormone levels in municipal bio-solids and animal manures, Lorenzen et al. (2004) reported the highest levels of steroid hormones (5.965 μg g\(^{-1}\) d.w.) in manure from finishing pigs, and the lowest level (0.43 ng g\(^{-1}\) d.w. in manure from steers) In a similar study, Lange et al. (2002a) reported maximum excretion of steroid hormones from swine and cattle manure as compared with other livestock sources in the European Union and the United States.

### 2.4 Environmental Fate and Transport of Steroid Sex Hormones

Recent times have seen reports of a number of instances of significant contamination of surface and ground water resources by manure-borne steroid hormones contributed by runoff effluent from agricultural fields having receiving manure applications. Kjær et al. (2007), investigating the transport of estrogenic hormones from manure-treated structured loamy soil to the tile drainage system, reported the leaching of manure-borne sex hormones, particularly high concentrations of E2, via macrospore flow. This occurred even three months after the manure’s application. They observed that even 11 months after the application of swine manure, both E2 and E1 were still detectable in drainage water at concentrations exceeding the LOEL. In a lysimeter experiment, Herman and Mills (2003) reported the frequent detection of estrogens in the vadose zone beneath manure-fertilized corn (Zea mays L.) Lægdsmand et al. (2009) reported the transport of estrogenic hormones to a depth of 1 m in pig slurry-treated loamy and sandy soil lysimeter monoliths, and the hormonal content of the leachate samples exceeded the LOEL of 10 ng L\(^{-1}\). Peterson et al. (2000) reported concentrations of E2 ranging from 6 to 66 ng L\(^{-1}\) in groundwater.
Shore et al. (1993) reported elevated aqueous concentrations of estrogens ranging from <0.5 to 5.4 ng L$^{-1}$ in a stream following the application of broiler litter to upstream agricultural land. Allen-King et al. (2002) reported the frequent detection of estrogens in the porous zone under a corn field receiving manure. Arnon et al. (2008) reported a deep vertical stratification of estrogens and testosterone in the unsaturated sediments below a CAFO waste lagoon down to of 45 and 32 m, respectively. This highlights the direct impact of a dairy farm on hormonal pollution of groundwater. Multiple reports have indicated the significant hormone contribution of topsoil-incorporated animal manure to leachate and surface runoff from agricultural fields. Jenkins et al. (2008) reported E2 concentrations of 38.7 to 196.3 ng L$^{-1}$ and testosterone concentrations of 3.3 to 7.4 ng L$^{-1}$ in the runoff collected from poultry-litter-amended fields following a rainfall event in four different cropped watersheds.

The environmental behavior and fate of progesterone is less well-known. A limited number of studies have reported on the occurrence of biologically active concentrations of progesterone in topsoil and runoff from agricultural fields treated with manure. Lange et al. (2002b) showed excretion of androgens and progestins at rates comparable to, or greater than, rates for estrogens. The incidence of these steroids has been reported in watersheds with significant animal agriculture. Kolpin et al. (2002) reported the contamination of 4.3% of streams in the United States with average and maximum progesterone concentrations of 0.11 μg L$^{-1}$ and 0.199 μg L$^{-1}$. Mansell et al. (2011), investigating the occurrence and pathways of six steroid hormones, including progesterone, in beef cattle feedlot runoff after simulated rainfall, detected biologically active concentrations of progesterone in soil and runoff samples. Bartelt-Hunt et al. (2012) investigating the occurrence of 16 endogenous and synthetic steroid hormones and metabolites in runoff from beef cattle feedlots and in manure and soil collected from feedlot surfaces, detected progesterone in both soil and manure samples. In runoff samples from feedlots average and maximum progesterone concentrations of 59.5 ng L$^{-1}$ and 570 ng L$^{-1}$, respectively. However, Schwarzenberger et al. (1996) indicated the key role of rapid metabolic transformation of progesterone in rapidly decreasing of its concentration and thereby influencing its environmental concentration. However, some potential stability of progesterone is expected given its previous detection in surface
water resources (Kolpin et al., 2002). Kolodziej and Sedlak (2007) estimated the contribution of rangeland grazing areas to steroids in surface waters by using measured concentrations and estimates of stream discharge to estimate total steroids loads. In another study, progesterone was detected in 5% of streams within the rangeland of roaming beef cattle, with a maximum concentration of 27 ng L$^{-1}$ (Kreinberg, 2012).

2.5 Environmental Behavior of Steroid Sex Hormones

The dissipation and environmental behavior of steroid sex hormones is a function of various physical, chemical and biotransformation processes, including sorption and desorption in the soil media (Ying et al., 2002), biodegradation, and photo degradation (Chowdhury et al., 2010).

2.5.1 Sorption/desorption

Sorption and desorption are the dominant phenomena in soil and are considered the main influence on the occurrence, fate and transport of organic contaminants in soil and sediments. These functions can regulate the aqueous concentration of these contaminants and as a result, affect leaching, environmental bioavailability and degradation of this class of toxic chemicals (Kookana, 2010). Sorption can be identified as “the accumulation or adherence of a substance to a solid particle either internally, at its surface or at an interface between the solid surface and the bathing solution” (Sparks, 2003). The physiochemical characteristics of the compound, including the molecular structure of the chemical (the type and number of functional groups, the polarity of the compound, the distribution of its charges and the solubility of the compound), the chemical characteristics of the sorbent and environmental characteristics such as the water potential, pH, temperature, ionic strength and redox potential are the most influential factors affecting the sorption/desorption behavior of the chemical (Loffredo and Senesi, 2006). Hydrophobic partitioning, hydrogen bonding, and nonspecific van der Waals interactions are the most common sorption mechanisms in soil media (Nguyen et al., 2005).

The sorption/desorption behavior of steroid sex hormones in soil-sediment matrices plays an important role in their environmental fate and transport. The factors affecting the
sorption rate, distribution and partitioning of steroid hormones in soil and water have been reported (Bonin and Simpson, 2007; Holbrook et al., 2004; Karickhoff et al., 1979; Sangsupan et al., 2006). The sorption and desorption isotherms of hormones can be determined by batch equilibrium experiments, while soil-column experiments provide a measure of the retention of a contaminant along the soil profile (Caron, 2011). The relative hydrophobicity of estrogens, with log $K_{ow}$ values ranging from 2.6 to 4.0 (Lai et al., 2002) is a good indication of the strong sorption affinity of these hormones. The positive correlation between the sorption of hydrophobic contaminants such as steroid hormones and the soil organic matter content was reported by Karickhoff et al. (1979). Many studies have investigated the sorption and dissipation of estrogens in soil and sediment and their partitioning affinity to clay minerals and organic colloids. Yamamoto et al. (2003) and Das et al. (2004) have shown that the sorption of hormones to clay or dissolved organic matter, known as mobile soil particles, can enhance steroid transport via runoff or leaching, and enhance bioavailability to solid phase bacteria (e.g., bacteria in biofilms) (Young and Borch, 2009). Yu et al. (2004) proposed that the sorption behavior of estrogens could be due to the reactive phenolic group in these hormones which can interact with humic acids or mineral surfaces via hydrogen and covalent bonds. However, a significantly enhanced desorption of polycyclic aromatic hydrocarbons (PAH) in low organic carbon soil was reported by Johnson and Amy (1995). Humic substances can increase the mobility of sorbed contaminants by acting as a mobile, or flowing, solid phase. However while humic substances have a sorption ability similar to the stagnant solid phase, they have a mobility potential similar or greater than the mobile aqueous phase (McGechan and Lewis, 2002).

Cox et al. (1997) proposed the association of hormones with dissolved or colloidal fractions of soil and/or manure as one possible explanation for hormones’ persistence and greater than expected mobility in soil. Fan et al. (2007) indicated that approximately 50-73% of estradiol and its metabolites were bound to humic substances, which represents more than 50% of the organic matter in the soil. The texture and physical properties of the soil should be considered as two other governing factors affecting the sorption-desorption behavior of hormones in soil. Casey et al. (2005) and Das et al. (2004)
reported that higher sand content in the upper soil profile would result in less sorption of E2 and thereby more E2 dissolved in the aqueous phase. This, in turn, would allow greater leaching of the hormones to lower soil depths. Non-hydrophobic sorption interactions of E2 through the interaction of the estrogen’s phenolic group with humic acids or mineral surfaces via hydrogen and covalent bonding have been hypothesized by Yu et al. (2004).

### 2.5.2 Biodegradation and abiotic transformation of estrogenic hormones

Steroid hormones can be a potential source of energy for bacteria in a redox (reduction/oxidation) reaction, or as a carbon source for cell growth (Donova, 2007; Korom, 1992). Biological and other physical-chemical processes play a significant role on the fate and degradation of E2 (Fan et al. (2007). Microorganisms transform these hormones by different mechanisms. The main bacterial transformation processes for estrogens and progesterone in natural soil are *Mycobacterium*, *Arthrobacter*, *Bacillus*, *Pseudomonas*, *Escherichia*, *Micrococcus*, and *Nectria* (Jenkins et al., 2004). These gram-positive bacteria influence the degradation process in several possible manners, though primarily by introducing hydroxyl groups at multiple positions along the steroid skeleton and sequential hydroxylations along carbons 22-27 of the sterol side chain (Young and Borch, 2009). Species of *Nocardia* and *Arthrobacter* are responsible for degradation of progesterone in soil (Donova, 2007; Young and Borch, 2009). The possible hydroxylation, steroid ketone reduction, double bond hydrogenation, and single bond dehydrogenation of these hormones by microalgae and fungi has also been reported (Faramarzi et al. (2008). The soil moisture content, redox conditions and temperature have been demonstrated to have an influence on microbial activity and thereby on the degradation of steroid sex hormones (Xuan et al., 2008). The degradation pattern of steroid hormones occurs in a pseudo-first order process.

Several laboratory studies, including that of Jacobsen et al. (2005), have investigated the degradation of estrogens under aerobic conditions in the presence of various organic matrices, including animal wastes and biosolids. They have shown that the major degradation pathway of hormones in soil is through microbial degradation and, as a result, is influenced by media temperature and pH. Jacobsen et al. (2005) found the initial
degradation rate of E2 to be 0.01 day\(^{-1}\). Colucci et al. (2001) demonstrated the persistence of steroid hormones to be significantly different at different soil temperatures. Colucci et al. (2001) reported a rapid biotransformation of E2 and E1 in agricultural soil, with a degradation rate ranging from 0.22 to 0.47 day\(^{-1}\), over a range of temperatures and moisture levels. This indicated a biotic and potential abiotic degradation of E2, but only a biotic degradation of E1. A rapid rate of microbial degradation of E2 (\(t_{1/2} = 0.17\) day) in non-sterilized soil was reported by Xuan et al. (2008). Das et al. (2004) conducted a series of fast-flow-velocity transport experiments under pulse-type and flow-interruption boundary conditions in columns packed with a surface soil in order to estimate the degradation coefficients of parent hormones and their metabolites. They estimated the degradation rate of E2 to range between 0.0003 to 0.075 hour\(^{-1}\). Colucci et al. (2001) studying the dissipation and mineralization rate of E2 and its primary metabolite E1 over a range of temperatures and moistures in laboratory microcosm incubations experiments, reported rapid dissipation of E2 in soil conditions typical of a temperate growing season. Rapid oxidation of E2 to E1 was observed in both autoclaved and non-sterile loam, silt loam, and sandy loam soils, suggesting a biological transformation. This contrasted with the observed stability of E1 in autoclaved soil.

2.6 Gap in Science

Although several detailed investigations of environmental pathways and ecotoxicology of high-toxicity-at-low-concentration steroid sex hormones in soil and water media have been conducted over the last decade, there is a paucity of knowledge regarding the environmental and aquatic fate and transport of progesterone. This lack of available knowledge in addressing the remediation of steroid hormones in the soil matrix and aquatic media, highlights the pressing need to conduct detailed studies in support of the development of economically feasible remediation techniques capable of reducing the environmental and biological consequences of hormonal pollution.

2.7 Biochar

An excess of biologically active toxins and high-risk organic and inorganic chemicals generated or exposed to the environment by anthropogenic activities has resulted in
unacceptable environmental risks and health threats to biota and humans. Environmentally acceptable alternatives are needed to overcome these unsustainable sources of contamination. Recently, the \textit{in situ} application of organic amendments to contaminated soils has been proposed as a multi-purpose and financially feasible alternative, whereby the engineering of natural processes can meet remediation needs, whilst providing conditions which promote plant growth and stimulate ecological restoration (Beesley et al., 2011).

Recent global environmental concerns regarding the role of anthropogenic activities in climate change, greenhouse gas emissions and contamination of fresh water resources, and the need to develop sustainable renewable energy sources have led to the development and production of renewable bioenergy. A highly touted alternative to non-renewable fossil fuel resources, the renewable-energy-focused technology of pyrolysis is defined as the thermo-chemical decomposition of biomass and biological residues (e.g. wood, poultry litter, crop residues, etc.) in the absence of oxygen (or its partial combustion in the presence of a limited oxygen supply). This process leads to the production of bio-oil, combustible gases and a fine-grained carbonaceous residue, termed biochar. Biochar has history reaching back to the late 19th century where a patch of dark, highly fertile soil known as \textit{terra preta} was found in the Amazon basin. This fertile high carbon content soil was created by the anthropogenic incorporation of high organic carbon-content by-product of biomass combustion in the absence of oxygen at relatively high temperatures (i.e., biochar) (Glaser et al., 2001). In contrast to the region’s un-improved and highly weathered oxisols, the biochar-amended soils showed greater fertility and improved yields (Winsley, 2007).

Biochar has an amorphous structure, containing nano-scale condensed aromatic rings forming a crystalline structure which, due to its high specific surface area and resistance to bio-decomposition, offers a strong and long-term sorption affinity for inorganic contaminants (e.g. heavy metals) and hydrophobic organic contaminants. Consisting of macromolecular particles with flexible pores (Figure 2.2; (Killops et al., 1993), biochar’s structure reflects its aliphatic, alicyclic, aromatic and hetero-aromatic backbone, cross-linked by metal ions or covalent bonds (Hayes et al., 1989). Its abundant mobile nuclei in
cross-linked structures and humic substances (Xia and Pignatello, 2001) allow biochar to adsorb small molecules into its matrix. Including a rudimentary pore structure (Lua et al., 2004) and a partially tar-like surface bearing mainly aromatic C, aliphatic C, carboxyl and carbohydrate residues, these, along with some volatile matter constitutes the essential components of biochar.

Fig 2.2 Scanning electron micrograph of slow pyrolysis biochar (scale represents 200 μm)

Pyrolysis, gasification, and combustion are major technologies for the thermal conversion of different biomasses to biochar. The yield and by products of each mechanism are presented in Fig 2.3. The pyrolysis technique can be classified according to pyrolysis conditions including the heating rate (fast, intermediate or slow). Slow pyrolysis which takes place at relatively low temperatures (300-500°C) with a long heating time and a heating rate of less than 10°K min⁻¹ generates a high solids (i.e., biochar) yield, whereas fast pyrolysis takes place over a short time at high temperatures (700-900 °C) with a heating rate exceeding 1000 °K min⁻¹ (Mohan et al., 2006). The differences in physical characteristics of the feedstock biomass and the quality of its organic materials, along with the pyrolysis conditions, lead to differences in the resultant biochar’s specific surface area, cation exchange capacity, ash content, volatile matter, organic carbon content, levels of different organic materials, particle size. Based on a proximate analysis a standard biochar’s nutrient composition consists of 172 to 905 g kg⁻¹ of actual carbon, 1.8 to 56.4 g kg⁻¹ of nitrogen, 2.7 to 480 g kg⁻¹ of Phosphorus (P) and 1.0 to 58 g kg⁻¹ of potassium (K) (McElligott, 2011). The transformations of biomass C to biochar C under
the pyrolytic process allows sequestration of about 50% of the initial C, compared to 3% after full aerobic combustion, less than 10–20% after 5–10 years of biological decomposition (Tejerina, 2010). The half-life of biochar C in soil exceeds 1000 years (Novak et al., 2009).

![Chart](image)

*Fig 2.3 Products and yields of different pyrolysis mechanisms (Bridgwater, 2003)*

Despite the relatively low nutrient content of biochar itself, part of its nutrient value in soil is attributable to its nutrition retention/leaching reduction capacity in soil, allowing greater plant nutrient uptake and higher crop yield (Chan et al., 2008). The basic elemental composition of various biochars is reported in Table 2.1.

Table 2.1 Summary of total elemental composition and pH range of different biochar (Tejerina, 2010)

<table>
<thead>
<tr>
<th></th>
<th>C (g kg⁻¹)</th>
<th>Total N (mg kg⁻¹)</th>
<th>NO₃⁻ – N (mg kg⁻¹)</th>
<th>NH₄⁺ – N (mg kg⁻¹)</th>
<th>P (g kg⁻¹)</th>
<th><em>Pₐ</em> (g kg⁻¹)</th>
<th>K (g kg⁻¹)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>172-905</td>
<td>1.7–78.2</td>
<td>0-2</td>
<td>0.2-73</td>
<td>0.015-11.6</td>
<td>1-58</td>
<td>6.2-9.6</td>
<td></td>
</tr>
</tbody>
</table>

*Pₐ: Average P*
Soil biological communities, comprising bacteria, archaea, fungi, algae, protozoa, nematodes, arthropods and a diversity of invertebrates can be influenced by the presence of biochar (Thies and Rilling, 2009), which alters the physical and chemical environment of the soil. The high internal surface area of biochar gives it the ability to absorb soluble organic matter, gases and inorganic nutrients in the topsoil and provide a highly suitable habitat for microbial communities to colonize, grow in and reproduce. The increased soil organic carbon which results from the high C content and stability of biochar plays a pivotal role in nutrient cycling leads to improvements in plant-available water reserves, soil buffering capacity and soil structure (Tejerina, 2010). From another perspective, the application of biochar influences the critical soil environmental factors affecting bacterial abundance, diversity and activity, including moisture, temperature and pH. Even at the highest biochar soil amendment rate (140Mg C ha⁻¹), Lehmann and Rondon (2006) observed crop yield improvements and no observable negative impacts compared to non-amended soil.

The use of biochar as a soil amendment has been shown to improve the soil physio-chemical properties, in particular increasing cation exchange capacity, structure, pore size distribution and density, nutrient availability/fertility, water holding capacity, water infiltration, and soil pH (Enders et al., 2012). Given biochar’s relatively low bulk density (~0.3 Mg m⁻³), its application to soil with a typical bulk density of 1.3 Mg m⁻³ can reduce the overall bulk density of the soil, resulting in more efficient growth media for plant (McElligott, 2011). A higher sorption affinity of biochar, compared to other forms of soil organic matter, has been reported for a range of organic and inorganic compounds. The same holds for biochar’s greater nutrient retention ability (Nguyen et al., 2007). Biochar’s increased number of surface carboxyl groups and consequently greater negative charge, which affords it a greater ability to sorb cations, could be due to the abiotic and biotic surface oxidation of biochar (Chen et al., 2008). Due to its carbonate content, biochar acts as a liming agent in acidic soil (Van Zwieten et al., 2010). The incorporation of biochar in agricultural soil results in a reduction in greenhouse gas emissions. Several studies have demonstrated a significant role of biochar as a soil amendment and in reducing nitrogen oxide (N₂O) and methane (CH₄) emissions (Troy et al., 2013). Beesley et al. (2011) described the positive effect of biochar’s retention
capacity on the mobility, bioavailability and toxicity of heavy metals and PAHs. Karami et al. (2011) in assessing the impact of biochar on the mobility of copper and lead, along with the plant uptake of heavy metals, found biochar to be effective in reducing the pore-water concentration of heavy metals. Recent laboratory studies (Yu et al., 2010; Zheng et al., 2010) have investigated the sorption capacity of biochar for different types of pesticides including triazine and acetamiprid. Spokas et al. (2009) reported an enhanced sorption ability of biochar-amended Minnesota soils for herbicides such as atrazine and acetochlor where biochar was applied as a soil amendment at sufficient levels to increase organic carbon content of the soil. The only investigation to evaluate the sorption capacity of biochar for estrogens was conducted by Sarmah et al. (2010). Despite recent documented evidence of biochar as a soil amendment has an effect on the adsorption-desorption and transport of pesticides and antibiotics, there is a paucity of studies addressing the retention ability of biochar as a novel remediation technique for agricultural-hormone pollution.
PREFACE TO CHAPTER 3

The review of current researches on the fate and transport steroid sex hormones indicated inadequate knowledge addressing the remediation of these hormones in the soil matrix and aquatic media. In this chapter, we evaluate the effectiveness biochar as a novel remediation technique for reducing hormonal pollution.

The sorption potential and desorption resistance of different biochars are dependent on the pyrolysis condition and feedstock. In this study, we evaluated the retention ability of two different types of biochar (variation based on production temperature, time and raw material) for reducing hormonal contamination in soil. Batch equilibrium experiments were used to determine the sorption-desorption isotherms of three female sex hormones (17β-estradiol, Estrone, Progesterone) in the presence of biochar.

The biochar with the better retention performance, from this study, was further used to study the fate and transport of manure-borne female sex hormones under field condition. This study will help us understand the role of biochar in mitigating surface water hormonal pollution. To the best of our knowledge, this study is the first investigation evaluating the sorption-desorption behavior of progesterone and reporting the desorption isotherms of all three female sex hormones in biochar-amended soil.

Research papers based on the chapter:

Chapter 3
Evaluation of the Remediation Potency of Slow and Fast Pyrolysis Biochar for the Reduction of Aquatic Hormonal Pollution

3.1 ABSTRACT

Over the last decade, biologically active natural and synthetic sex hormones, and their structural analogues, have contributed to major environmental safety and sustainability issues. Despite the presence of these hydrophobic hormonal compounds in agricultural surface and subsurface water resources, there is a paucity of studies addressing the remediation of these sex hormones in aquatic media. This study investigated the potential remediation capacity of slow and fast pyrolysis biochars, as soil amendments, for reducing the hormonal pollution in soil. The sorption affinity and desorption resistance of each biochar for three steroid sex hormones, 17β-estradiol (E2), estrone (E1) and progesterone (P), was evaluated by a multi-concentration, batch equilibrium experiment. Both the sorption and desorption isotherms were found to be non-linear in the soil-biochar treatments for all three hormones. A significant difference was found between the concentration-dependent, effective sorption coefficient ($k_{d_{eff}}$) of the soil-biochar treatments and soil treatments indicating a strong sorption capacity and significant desorption resistance of biochar as a soil amendment. This assessment confirms the hypothesis of the effectiveness of biochar’s sorption capacity for manure-borne hormones to lessen the leaching of these compounds through the soil profile and into drainage water.

Keywords. Sorption, desorption isotherm, 17β-estradiol, estrone, progesterone, biochar

3.2 Introduction

3.2.1 Emerging contaminants

In the last decade, many environmental studies have focused on the prevalence, fate, transport pathways and ecotoxicology of an obscure and biologically active category of emerging contaminants, which have become a major ecological and environmental safety issue and a biological threat to human health. These toxic and high-risk organic contaminants, include a wide variety of personal-care products, industrial reagents, nano-
particles, surfactants (Farré et al., 2008), ectoparasiticides, mycotoxins, heavy metals and dioxins (Khan et al., 2008). These contaminants have not been regulated according to environmental and health guidelines (e.g. water-quality monitoring guidelines) and are poorly documented for their toxicological and biological risks (Schriks et al., 2010). Pharmaceuticals, veterinary drugs (e.g. antibiotics), and endocrine disrupting compounds (EDCs) are of major concern due to their chronic toxicity and other adverse, long-term health issues (e.g. carcinogenicity, mutagenicity or teratogenicity) at extremely low concentrations (e.g. 10-100 ng/L)(Choi et al., 2004; Hanselman et al., 2003). Another challenging aspect of these chemicals results from their high toxicity, persistence and strong estrogenicity of their metabolites in environmental media.(Farré et al., 2008)

3.2.2 Endocrine disrupting compounds

EDCs, including a variety of synthetic and industrial compounds are known to modulate the functions of the endocrine system by mimicking, counteracting, altering or interfering with the metabolism and biosynthesis of endogenous hormones.(Colucci et al., 2001; Sonnenschein and Soto, 1998). Among EDCs, the natural and synthetic steroidal sex hormones have attracted the attention of scientists due to their detrimental and carcinogenetic effects; they influence physiological functions by interfering with the normal endocrine system and resulting in sexual and reproductive abnormalities and intersexuality in the aquatic environment. The potential high-risk steroid sex hormones are 17β-estradiol (E2) and its primary metabolites, estrone (E1), progesterone, testosterone and cortisol.

Extensive evidence of endocrine disruption has been reported (Balabanič et al., 2011; Colborn et al., 1993; Diamanti-Kandarakis et al., 2009; Söffker and Tyler, 2012). Lange et al. (2011) demonstrated that exposure to EDCs contained in wastewater treatment effluent discharges, led to a high percentage of feminization of the roach, Rutilus rutilus, living in UK(United Kingdom) rivers. Several other cases of aquatic biological disruption cause by steroid hormones have been indication in large number of environmental studies. The feminization of male fish or the masculinization of female fishes (Orlando et al., 2004; Soto et al., 2004; Vajda et al., 2011; Vajda et al., 2008) or reproductive biology alteration of wild fathead minnows (Pimephales promelas) and rainbow trout at aquatic
estradiol concentration, as low as 1-10 ng/L and 25-50 ng/L of E1 (Fang et al., 2003; Young and Borch, 2009), are good evidence of hormonal environmental contamination. Partridge et al. (2010) point out that endocrine chemical disruptors have the potential to alter not only the primary sexual traits and reproduction functions but also secondary sexual traits, which can affect mating dynamics. Since the female sex hormones have been detected in concentration above their lowest observation effect level (LOEL), 10 ng/L (Shore and Shemesh, 2003), and due to their higher potential physiological disorder at lower concentration than other steroid hormones, they have become the major concern among all the EDC’s. The free and conjugated form of female hormones are environmentally stable with high biotransformation potential (Shore and Shemesh, 2003).

Although hormones are produced naturally by humans and livestock (Combalbert and Hernandez-Raquet, 2010), environmental exposure to these hormones are through the expanding anthropogenic activities, wastewater plant (WTPs) discharges and agricultural land management, specifically the application of animal manure and other bio-solids (e.g. sewage sludge) on agricultural land. Considerable amounts of animal wastes, generated by expanding anthropogenic activities (e.g., concentrated animal feeding operations), are applied to soil as a soil-amendment and as a nutrient source to boost soil fertility. Although it is recognized as a prevalent organic agricultural soil-amendment and soil fertility booster in North America, swine and poultry manure has been a major source of non-point pollution of natural steroidal sex hormones, including E2, E1 and progesterone. Shore and Shemesh (2003) reported the hormone content of different animal manures, including the E2 concentration of diary (239±30 μg/kg), swine (1215±275 μg/kg) and (33±12 μg/kg) (Schuh et al., 2011).

3.2.3 Environmental fate of sex hormones

Various physical, chemical and biotransformation processes such as sorption and desorption in the soil media (Casey et al., 2005; Ying and Kookana, 2009), biodegradation or photo degradation (Chowdhury et al., 2010; Khanal et al., 2006; Lee et al., 2003; Zhang et al., 2007), can influence the environmental fate and transport of manure–borne sex hormones. These problematic hormonal compounds are generally weakly acidic and hydrophobic and consequently, show a high affinity for sorption in
soil. Several studies have shown that they are present in surface and ground water (Arnon et al., 2008; Kjær et al., 2007; Kolodziej et al., 2004). The US (United Stare) Geological Survey found detectable concentrations of steroid hormones at 21% to 139% in surface water and streams in the US. The elevated aqueous concentration of estrogens from <0.5 to 5.4 ng L\(^{-1}\) in the stream following the application of broiler litter to the upstream agricultural land was reported by Shore et al. (1993). In the investigation conducted by Finlay-Moore et al. (2000) and Nichols et al. (1997), run-off concentrations of 100-2500 ng L\(^{-1}\) of E2 was found after the application of poultry manure. The presence of these hydrophobic, high-toxicity-at-low-concentration hormonal compounds in agricultural surface and subsurface (leachate) runoff has brought them to the forefront as environmental issues that need to be addressed through controlled studies. Several studies have investigated the occurrence, fate and transport and the environmental behavior of hormones such as sorption-desorption and biodegradation of hormones in soil and aquatic matrices (Combalbert and Hernandez-Raquet, 2010; Hansen et al., 2011; Jenkins et al., 2008; Sangsupan et al., 2006; Steiner, 2009; Ternes et al., 2002). However, there is a paucity of studies addressing the remediation of these sex hormones in aquatic media.

### 3.2.4 Biochar

The in-situ application of organic carbonaceous materials as a soil amendment is considered a multi-purpose contaminant retention technique to improve soil quality, crop performance and ecological conditions. This new remediation technique has been proposed as a financially-feasible approach to engineer the natural process in order to fulfill an environmental remediation requisite (Beesley et al., 2011). The application of different organic industrial or animal residues as soil amendments have the potential to reduce the environmental, ecological and biological risks and long term adverse health effects posed by exposure to emerging contaminants in surface water and soil media (Beesley et al., 2011). The strong sorption affinity of carbon rich amendments, such as black carbon, activated carbons and biochar for polycaromatic hydrocarbons and other categories of organic contaminants may make it possible to use them as a novel bio-remediation technique. The remediation potential of these carbonaceous materials to reduce the bioavailability and bioaccumulation of organic contaminants has been
demonstrated recently (Brändli et al., 2008; Cho et al., 2009). Recent global environmental concerns for climate change, greenhouse gas emissions and contamination of fresh water resources due anthropogenic activities and the need for sustainable renewable energy sources have led to the production of renewable bioenergy.

The thermo-chemical decomposition of biomass and biological residues (e.g. wood, poultry litter, crop residues, etc.) in the absence of oxygen (or partially combusted in the presence of a limited oxygen supply) is known as pyrolysis and leads to the production of bio-oil, combustible gases and a fine-grained carbonaceous residue, named biochar. The biomass itself is considered as the source of energy for the thermal process (Bridgwater, 2003). The pyrolysis technique can be classified according to different methods based on the pyrolysis conditions including the heating rate where it may be a fast, intermediate or slow pyrolysis. The slow pyrolysis would take place at relatively low temperatures (300-500 °C) with a long heating time and a heating rate less than 10 K/min with high yields of solids, i.e., biochar, whereas fast pyrolysis takes place over a short time at a high temperature (700-900 °C) with a heating rate of more than 1000 K/min (Mohan et al., 2006). Improving the soil physio-chemical properties including the increased cation exchange capacity (CEC), more nutrient availability and better fertility, increased pH levels, higher water holding capacity water infiltration and a better nutrient availability (Enders et al., 2012; Novak et al., 2009; Oguntunde et al., 2004; Van Zwieten et al., 2010; Warnock et al., 2007) have been reported as the potential effects of biochar as a soil amendment.

Biochar has an amorphous structure, containing nano-scale condensed aromatic rings with a crystalline structure and consequently, which offers a strong sorption affinity for inorganic contaminants (e.g. heavy metals) and hydrophobic organic contaminants due to its high specific surface area and resistance to bio-decomposition. The incorporation of biochar in agricultural soil resulted in a reduction in greenhouse gas emissions. Several studies (Rondon et al., 2005; Spokas et al., 2009; Troy et al., 2013) demonstrated the significant role of biochar as a soil amendment or resulted in the reduction of nitrogen oxide (N₂O) and methane (CH₄) emissions. Beesley et al. (2011) described the positive effect and retention capacity of biochar on the mobility, bioavailability and toxicity of
heavy metals and PAHs. Karami et al. (2011) assessed the impact of biochar on the mobility of copper and lead and plat uptake of heavy metals, where biochar was effective in reducing the pore-water concentration of heavy metals. Recent laboratory studies (Chun et al., 2004; Smernik, 2009; Yu et al., 2010; Zheng et al., 2010) investigated the sorption capacity of biochar on different types of pesticides including triazine and acetamiprid. The only investigation to evaluate the sorption capacity of biochar for estrogens was conducted by Sarmah et al. (2010). The main objective of this study was to evaluate the retention ability of two types of biochars (fast and slow pyrolysis biochar) as a novel approach for reducing soil and water pollution by the three major manure-borne steroidal sex hormones (E2, E1 and progesterone) via batch equilibrium studies. Based on the recent documented evidence of biochar as a soil amendment on the adsorption-desorption and transport of pesticides and antibiotics, the results of our study could lead to a novel remediation technique for agricultural-hormone pollution. To the best of our knowledge, this study is the first investigation evaluating the sorption-desorption behavior of progesterone and reporting the desorption isotherms of female sex hormones in a biochar-amended soil.

3.3 Methodology

3.3.1 Chemicals and apparatus

The analytical chemical standards for all three female sex hormones, E2 (>98% purity) and its primary metabolite, E1 (>99% purity) and progesterone (>98% purity) were purchased from Sigma Aldrich (St. Louis, MO, USA). The anhydrous calcium chloride standard was provided by Science lab, Houston, Texas, USA. The physiochemical properties and the chemical structure of these steroid hormones are shown in Table 3.1 and Table 3.2. High performance liquid chromatography grade Acetonitrile, which was used both, as a solvent and for the mobile phase, was purchased from Fisher science. The de-ionized water, provided from Milli-Q (Billerica, MA) system with resistivity more than 18 MΩ, was used as the mobile phase in the analytical analysis of samples, as a solvent in the desorption bath equilibrium experiments and in all steps of the Standard solution preparation. All stock solutions and diluted lower concentrations of the three hormones, used to obtain the calibration curves and the batch equilibrium experiments,
were prepared using these chemical standards. Following the dilution of a specific calculated volume of progesterone and estrogen standard stock solutions (100 mg L\(^{-1}\)) in HPLC grade acetonitrile, the seven lower concentrations of the standard solution (5, 1, 0.5, 0.1, 0.05, 0.01, 0.005 and 0.003 mg L\(^{-1}\)) for obtaining the calibration curves and to spike the soil samples in the bath equilibrium experiments were prepared. For each experiment, fresh stock solutions and standards were prepared.

Table 3.1 Physicochemical Properties of Estrogens (Colucci et al., 2001; Young and Borch, 2009; Yu et al., 2004)

<table>
<thead>
<tr>
<th>Chemical Structure</th>
<th>17(\beta)-estradiol (E2)</th>
<th>Estrone (E1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular formula</td>
<td>(\text{C}<em>{18}\text{H}</em>{22}\text{O}_2)</td>
<td>(\text{C}<em>{18}\text{H}</em>{22}\text{O}_2)</td>
</tr>
<tr>
<td>Molecular weight (g/mol)</td>
<td>272.4</td>
<td>270.4</td>
</tr>
<tr>
<td>Water solubility (mg L(^{-1})) @ 25 °C</td>
<td>3.6</td>
<td>0.8-12.4</td>
</tr>
<tr>
<td>Vapor pressure (mm Hg) (3\times10^{-8})</td>
<td>3\times10^{-8}</td>
<td>3\times10^{-8}</td>
</tr>
<tr>
<td>(pK_a)</td>
<td>10.1</td>
<td>10.7-10.8</td>
</tr>
<tr>
<td>(\log K_{OW})</td>
<td>3.94</td>
<td>3.43</td>
</tr>
<tr>
<td>(\log K_{OC})</td>
<td>3.34</td>
<td>3.2</td>
</tr>
<tr>
<td>(t_{1/2}) (d)</td>
<td>0.3-0.5</td>
<td>0.6-1.7</td>
</tr>
<tr>
<td>Relative estrogenic potency (EEF)</td>
<td>1</td>
<td>0.02-0.3</td>
</tr>
</tbody>
</table>

3.3.2 Biochar characteristics

Dynamotive biochar and BlueLeaf biochar were provided by BlueLeaf Inc, Drummondville, Quebec, Canada. The Dynamotive biochar is produced through the fast pyrolysis of hardwood waste material at 750 °C and the BlueLeaf biochar is the product of the slow pyrolysis of soft wood at 450 °C. The detailed physical, chemical and elemental characterization of each biochar is provided by the soil control lab of Control Laboratories Inc., Watsonville, California, USA. The detailed characterization of each type of biochar is presented in Table 3.3 to Table 3.7.
Table 3.2 Physico-chemical Properties of Progesterone (Colucci et al., 2001; Young and Borch, 2009; Yu et al., 2004)

**Progesterone (P)**

**Chemical Structure**

![Chemical Structure of Progesterone](image)

**Molecular formula**

C_{21}H_{30}O_2

**molecular weight (g/mol)**

314.46

**Water solubility (mg L\(^{-1}\) @ 20 °C)**

8.81

**Vapor pressure (mm Hg)**

1.3 × 10\(^{-6}\) mm Hg at 25°C

**log \(^{\text{OW}}\)**

3.67-3.87

**\(t_{1/2}\) (d)**

35.8-55.13 hrs

**log \(^{\text{RBA}}(1)\)**

-0.7

(1) RBA: relative binding affinities (RBA) for androgen and estrogen receptors

Table 3.3 Proximate Analysis of Dynamotive and Blueleaf Biochar

<table>
<thead>
<tr>
<th>Dry weight constitution</th>
<th>Carbon (C) (%)</th>
<th>Hydrogen (H) (%)</th>
<th>Nitrogen (N) (%)</th>
<th>Sulfur (S) (%)</th>
<th>Oxygen (O) (%)</th>
<th>Total Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dynamotive</td>
<td>74</td>
<td>3.2</td>
<td>0.42</td>
<td>0.02</td>
<td>12.6</td>
<td>9.8</td>
</tr>
<tr>
<td>BlueLeaf</td>
<td>77</td>
<td>2.2</td>
<td>0.56</td>
<td>0.02</td>
<td>3</td>
<td>17.3</td>
</tr>
</tbody>
</table>

Table 3.4 Particle size distribution analysis of Dynamotive and BlueLeaf biochars

<table>
<thead>
<tr>
<th>Particle Size Distribution</th>
<th>Blue Leaf Biochar</th>
<th>Dynamotive Biochar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.15 mm</td>
<td>2.9</td>
<td>18.8</td>
</tr>
<tr>
<td>0.15-0.18 mm</td>
<td>4.4</td>
<td>21.6</td>
</tr>
<tr>
<td>0.18-0.25 mm</td>
<td>4.9</td>
<td>21.5</td>
</tr>
<tr>
<td>0.25-0.425 mm</td>
<td>3.9</td>
<td>24.3</td>
</tr>
<tr>
<td>0.425-0.850 mm</td>
<td>9.4</td>
<td>11.4</td>
</tr>
<tr>
<td>0.85-2 mm</td>
<td>22</td>
<td>2.4</td>
</tr>
<tr>
<td>2-6.3 mm</td>
<td>13.9</td>
<td>0</td>
</tr>
<tr>
<td>6.3-9.5 mm</td>
<td>18.4</td>
<td>0</td>
</tr>
<tr>
<td>9.5-16 mm</td>
<td>6.9</td>
<td>0</td>
</tr>
<tr>
<td>16-19 mm</td>
<td>13.2</td>
<td>0</td>
</tr>
<tr>
<td>&gt; 19 mm</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Based on the detailed proximate analysis of Dynamotive and Blueleaf biochar, the volatile matter content of the fast pyrolysis biochar is calculated about 17% whereas the slow pyrolysis biochar consists of less than 6% volatile matter content. A higher fixed carbon content is reported for the slow pyrolysis biochar. The significantly lower oxygen content of slow biochar (3%) as compared to the fast biochar (12.6%) can be considered as an indication of the higher specific surface area of the slow biochar. The particle size distribution analysis of each biochar demonstrates the structural difference between the two biochars. The blueleaf biochar contains mainly larger particles with a diameter from 2 to 19 mm, as compared to Dynamotive biochar. This would indicate a higher heterogeneity for the slow biochar with higher macro-porosity. Almost 19% of the particles in the Dynamotive biochar structure have a diameter smaller than 0.15 mm. More than 50% of the particles fall in the area of 0.15 to 0.85 mm. Briefly, the fast pyrolysis biochar has a fine-powdered structure whereas the slow pyrolysis biochar has a larger particle structure. The reported pH values for slow and fast pyrolysis biochar were 9.35 and 8.65, respectively, indicating the basic nature of biochar samples, which could be due to the high ash content of both biochars. This basic characteristic of biochar can be considered as a fundamental remediation potential for acidic soil conservation and improvement.

Table 3.5 Structural analysis of Dynamotive and Blue Leaf biochar

<table>
<thead>
<tr>
<th></th>
<th><strong>Dynamotive Biochar</strong></th>
<th><strong>Blue Leaf Biochar</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Ash</td>
<td>9.80%</td>
<td>17.30%</td>
</tr>
<tr>
<td>Organic Carbon</td>
<td>74%</td>
<td>77%</td>
</tr>
<tr>
<td>Inorganic Carbon</td>
<td>0.12%</td>
<td>0.50%</td>
</tr>
<tr>
<td>Hydrogen/carbon (H:C)</td>
<td>0.55 (molar ratio)</td>
<td>0.34 (molar ratio)</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>3.40%</td>
<td>2.20%</td>
</tr>
<tr>
<td>Total nitrogen (N)</td>
<td>0.42%</td>
<td>0.56%</td>
</tr>
<tr>
<td>Liming (neut. value)</td>
<td>12.9% CaCO₃</td>
<td>12.6% CaCO₃</td>
</tr>
<tr>
<td>Liming (carbonate value)</td>
<td>0.98% CaCO₃</td>
<td>3.8% CaCO₃</td>
</tr>
<tr>
<td>Activity Iodine or Butane</td>
<td>4.4g/100g</td>
<td>9.6g/100g</td>
</tr>
</tbody>
</table>

*The H/C ratio indicates the degree of carbonization of biochar*
The evaluation of the basic soil enhancement properties of both biochars demonstrated a different concentration of minerals such as potassium (K), phosphorous (P), ammonia (NH₄-N), nitrate (NO₃-N), which could be due to different feed stocks, elemental contents and pyrolysis conditions such as heating temperature and heating rate. Both biochars have a similar liming potential of almost 13% of CaCO₃. The carbon content of the slow-pyrolysis biochar (77%) was slightly higher than the fast-pyrolysis biochar (74%). The slow biochar also contains a higher percentage of ash (%18) than the fast biochar (%10). The H/C ratio for slow biochar is 0.34%, indicating the high aromatic structure of the biochar with more organic carbon in texture, whereas the H/C ratio of the fast biochar was 0.55 %. Un-significant Nitrogen content was observed between the two biochars where the slow biochar contains 0.56% nitrogen and the fast biochar contains 0.42% nitrogen.
One of the common ways to describe the sorption characteristic of carbonaceous materials is to determine the amount of adsorbed specific chemical per unit weight of that carbonaceous media; this would be represented by the iodine activity number or butane activity which indicates the adsorption ability of biochar to trap butane from dry air under specified conditions. The butane activity represents the micro pore volume of the porous zone of biochar. In contrast to the iodine activity number, the butane activity number does not necessarily provide information regarding the absolute or relative effectiveness of the biochar. The higher butane activity number for the slow pyrolysis biochar (9.6g/100g) when compared to the fast biochar (4.4 g/100g) indicates more available micro pore volume and a higher potential sorption affinity for the slow biochar (Sean J. Bailey, 2010). The saturated hydraulic conductivity of the slow and the fast pyrolysis biochars was measured using the constant head method, described by Bowles (1992), it was 0.26 m/sec for slow pyrolysis biochar and 0.426 m/sec for fast pyrolysis biochar.

### 3.3.3 Soil characteristics

The soil chosen for the laboratory pilot study was a sandy soil (with 92.2 % sand content) of the Ste-Amable complex, Ferro-Humic podzol, which was brought from the first 10 cm of the outdoor lysimeters assigned for the field evaluation of the research. The lysimeter field was located on the Macdonald campus of McGill University, Ste-Anne-De-Bellevue, Quebec. The physical characteristics of the soil are presented in Table 3.8.

<table>
<thead>
<tr>
<th>Soil texture</th>
<th>Physical Properties</th>
<th>Sand %</th>
<th>Saturated hydraulic conductivity</th>
<th>Bulk density (kg/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand</td>
<td></td>
<td>92.2</td>
<td>3.68</td>
<td>1350</td>
</tr>
<tr>
<td>Silt</td>
<td></td>
<td>4.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clay</td>
<td></td>
<td>3.5</td>
<td>2.97 %</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.8 Physical characteristics of the soil (Adopted from Fan (2009))

3.4 Experimental Set up

3.4.1 Sorption-desorption experiments

The remediation potential and sorption efficiency of the two types of biochars (fast and slow pyrolysis biochars) was assessed through a set of laboratory batch equilibrium
experiments to evaluate and characterize the sorption/desorption behavior of the three female sex hormones and their mobility in the presence of biochar.

A modified protocol based on the equilibrium sorption experiment procedure used in the studies by Lee et al. (2003), and Sarmah et al. (2010), was applied. As demonstrated by Walker and Watson (2010), due to the statistically significant effect of the laboratory materials and filters e.g. plastic or stainless steel on the adsorption affinity of the steroid hormones, for all major steps of the batch experiment, glass-made materials was chosen to avoid a concentration deviation due to the sorption of compounds to the tubes and other experimental materials. Each batch included three different treatments, soil (S), soil and slow biochar (SSB) and soil and fast biochar (SFB). The soil was air-dried for 24hrs and was passed through a 2mm sieve and homogenized. For the preparation of each treatment, Pyrex glass centrifuge tubes with Teflon–lined screw caps were used.

The stock solution of each hormone was prepared in pure HPLC-grade acetonitrile to make sure that the compounds were fully dissolved. Following the indicated measured values of sex hormones in swine and poultry manure in several studies (Bevacqua et al., 2011), six concentrations of progesterone and estrogens (5, 1, 0.5, 0.1, 0.05, 0.01 mg L⁻¹ prepared in 0.005 M CaCl₂) were chosen as the initial spiking concentrations to measure the sorption isotherms for each treatment. 30 mL of each concentration of sex hormones was added to accurately weighed 2 g of soil as a control treatment and two other biochar–containing treatments in the Pyrex glass centrifuge tubes with Teflon–lined screw caps. Each soil-biochar treatment contained 1% (w/w) of biochar, based on the integration of the biochar at the first 10 cm of the topsoil with an application rate of 10 t/ha (Sarmah et al., 2010). All three treatments (S, SSB, SFB) were replicated three times in the batch experiment, after covering the samples with aluminum foil. The treatments containing progesterone standards were placed on a reciprocation shaker and agitated for 48 hours to reach equilibrium. The treatment samples with E2 and E1 were kept on the shaker for 24 hours to reach the equilibrium. All samples were kept at 25±0.5 °C. After reaching the equilibrium time for each compound, in order to separate the soil sample supernatants, all Pyrex glass tubes were centrifuged at 3230 rpm (1750 G) for 12 minutes and the supernatants were transferred to another set of acid washed and sterilized Pyrex tubes. 1.5
mL of each replicate of each treatment’s aliquot were filtered by 0.22 μm sterile filters and transferred into the amber HPLC vials and analyzed.

Using the indirect method of mass balance calculation, the concentration of hormones in the filtrates of each sample was measured after equilibrium and subtracted from the initial concentration of the standards. The amount of hormones sorbed to the soil and the soil and biochar mixture were determined. After carefully removing all supernatants in the last step of the sorption test, 30 mL of purified Milli-Q water was added to each treatment sample to accomplish the desorption test. The centrifuge tubes were covered with aluminum foil again and were agitated for 24 hours. The samples were centrifuged, as described in the sorption test, and 1.5 mL of the supernatants were sub-sampled and filtered as described in the previous section and kept in the amber HPLC vials to be analyzed and to determine the desorbed concentration of each hormone in the different treatments.

3.5 Instrumentation and Analysis

High performance liquid chromatography (HPLC) analysis was carried out to detect and quantify the progesterone, and the other two estrogen hormone standards. The analysis and concentration quantification of these hormones in the soil extracts and the aliquots were performed by a quaternary pump LC system from Agilent 1100 series technologies (Germany) equipped with a diode array-ultraviolet detector.

3.5.1 Separation, identification and quantification of progesterone, E2, E1

3.5.1.1 Progesterone

All concentrations of the progesterone standard solutions were prepared in HPLC-grade acetonitrile. The detection and concentration tracing of the progesterone was carried out through reverse-phase NOVA Pac C-18 column (300×3.9 mm), and a DAD detector at the representative response of 240 nm with the retention time of 10 min. A volumetric ratio of 40% of purified Milli-Q water and 60% HPLC-grade acetonitrile, with flow rate of 1 mL/min, were applied to the system as the mobile phase, with the volume of injection of 100 μL for isocratic analysis of progesterone.
3.5.1.2 17β-estradiol and Estrone

The specific concentration of the estrogen hormones standard solution was prepared in 50/50 (V/V) purified Milli-Q water and HPLC-grade acetonitrile. The detection and concentration tracing of these two estrogenic hormones were carried out through a Zorbax Eclipse Plus C18 column (150×4.6 mm) with particle size of 5 μm (Agilent, Santa Clara, CA). Since each HPLC column can be used for certain number of samples, a new column was used for detection of estrogen hormones in soil samples. The best detector response for identification of both E2 and estrone was at 200 nm with the retention time of 8 min (17-βestradiol) and 13 min (Estrone). The mobile phase used in this process was a volumetric mixture of a ratio of 60% of purified Milli-Q water and 40% HPLC-grade acetonitrile, with a flow rate of 1 mL/min with the injection volume of 100 μL for isocratic analysis of estrogens.

Prior to each analysis, the mobile phase was filtered by 0.45 μm membrane filters and degassed. The column temperature was kept constant at 25 °C during the analysis. To identify the retention time and the peak geometry of each compound, the injection of pure standards of each hormone, with a different range of concentrations (5-0.001 mg L⁻¹), was performed. By using the external standard peak areas from a chromatogram, at specific concentrations, a seven-point calibration curve equation for each compound was determined based on a linear regression in Excel (Microsoft Office Software). Furthermore, in order to determine the concentration of hormones in the experimental samples, the peak’s areas, which were obtained from the each sample chromatogram, were compared with the hormones standards peak’s areas and the concentrations were quantified by standard calibration curves. The detection response of the instrument to the lowest concentration of each hormone was different. The limit of detection for the progesterone was 0.001 mg L⁻¹, 0.003 mg L⁻¹ for E2 and E1.

3.6 Analysis and Modeling of the Sorption-Desorption Isotherms

3.6.1 Sorption isotherms

The amount of each hormone sorbed in each treatment (Cs, μg/g) was calculated by the mass-balance difference between the initial and analytically measured equilibrium concentration (Ceq, mg/L) of each compound. The sorption isotherm of each hormone
was determined by fitting the batch equilibrium sorption data to the Freundlich sorption model:

\[
C_{ads}^{s(eq)} = K_f C_{aq}^{ads} n
\]

Where \( C_{ads}^{s(eq)} \) is the content of the hormone adsorbed to each treatment at sorption equilibrium (μg/g) and \( C_{aq}^{ads} \) is the mass concentration of the hormones in the aqueous phase at adsorption equilibrium (mg/L). \( K_f \) (mg\( ^{1-n} \) (L\( ^n \) g\(^{-1} \)) is the Freundlich adsorption coefficient and \( n \) is the dimensionless Freundlich constant and generally ranges between 0.7-1.0, indicating non-linearity of the sorption data. Regression analysis using the log transformation form of the empirical Freundlich equation was used to quantify the Freundlich adsorption coefficient (\( K_f \)). The sorption isotherms are presented in Table 3.9.

3.6.2 Desorption-isotherms

For each treatment, the desorption behavior of the three hormones were investigated by comparing the amount of each hormone desorbed to the adsorbed amount under equilibrium conditions. The hormone content, remaining adsorbed on each treatment at desorption equilibrium is quantified as follows:

\[
C_{des}^{s(eq)} (\mu g/g) = (m_{ads(eq)} - m_{des(aq(eq))}) / m_{(soil)}
\]

Where \( m_{des(aq(eq))} \) is mass of hormone in the solution at adsorption equilibrium (desorbed from soil) at desorption equilibrium (μg) and \( m_{ads(eq)} \) is mass of hormone sorbed on soil at sorption equilibrium (μg). The desorption isotherms were determined by fitting the desorption equilibrium data to the Freundlich equation, relating the amount of hormones remaining adsorbed on soil and soil-biochar treatments to the concentration of each hormones in the solution at equilibrium:

\[
C_{des}^{s(eq)} = K_f C_{aq}^{des} n
\]
Where $C_{s(eq)}^{des}$ is the content of the hormone adsorbed to each treatment at desorption equilibrium (μg/g) and $C_{aq}^{des}$ is the mass concentration of the hormones in the aqueous phase at desorption equilibrium (mg L$^{-1}$) and $K_f$ (mg$^{1-n}$ (L) $^n$ g$^{-1}$) is the Freundlich desorption coefficient.

The log transformation form of the empirical Freundlich equation was used to calculate the desorption coefficient. In contrast to the linear nature of sorption and desorption isotherms for the soil treatment, the isotherms for the soil-biochar treatments were nonlinear, and therefore, this incoherence linearity of the isotherms makes it difficult to compare the sorption /desorption coefficient ($K_d$) and the organic carbon normalized sorption coefficient ($K_{oc}$) between the soil and the soil-biochar treatments due to their concentration-dependency. Therefore, in order to compare the sorption and desorption isotherms, the concentration-dependent effective sorption distribution coefficient, $K_d^{eff}$ (L/kg) = ($K_f / C_w^{n-1}$) (Hildebrand et al., 2006) was calculated at the single solution equilibrium concentration of $C_{aq}^{ads} = 0.5$ (mg L$^{-1}$). The log$_{10}$ of the organic carbon normalized partitioning coefficient log $K_{oc}$ was calculated where the $K_{oc} = (K_f / C_w^{n-1}) / f_{OC}$ at $C_{aq}^{ads} = 0.5$ mg L$^{-1}$, and $f_{OC}$ is the organic carbon content of the soil.
Table 3.9 Sorption isotherm fitted to the Freundlich equation for 17β-estradiol, Estrone, Progesterone

**Sorption Isotherms**

### 17-βestradiol (E2)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$K_f$</th>
<th>$n$</th>
<th>$R^2$</th>
<th>$K_{d_{eff}}$</th>
<th>log $K_{oc}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil + 1% Slow Biochar</td>
<td>23.211</td>
<td>0.942</td>
<td>0.981</td>
<td>24.170</td>
<td>3.147</td>
</tr>
<tr>
<td>Soil +1% Fast Biochar</td>
<td>16.073</td>
<td>0.949</td>
<td>0.971</td>
<td>16.654</td>
<td>2.985</td>
</tr>
<tr>
<td>Soil with Biochar treatment</td>
<td>0.757</td>
<td>1.048</td>
<td>0.961</td>
<td>1.014</td>
<td>1.770</td>
</tr>
</tbody>
</table>

### Estrone (E1)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$K_f$</th>
<th>$n$</th>
<th>$R^2$</th>
<th>$K_{d_{eff}}$</th>
<th>log $K_{oc}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil + 1% Slow Biochar</td>
<td>18.493</td>
<td>0.919</td>
<td>0.986</td>
<td>19.567</td>
<td>3.056</td>
</tr>
<tr>
<td>Soil +1% Fast Biochar</td>
<td>12.706</td>
<td>0.942</td>
<td>0.989</td>
<td>13.223</td>
<td>2.885</td>
</tr>
<tr>
<td>Soil with Biochar treatment</td>
<td>0.815</td>
<td>1.141</td>
<td>0.996</td>
<td>0.739</td>
<td>1.633</td>
</tr>
</tbody>
</table>

### Progesterone (P)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$K_f$</th>
<th>$n$</th>
<th>$R^2$</th>
<th>$K_{d_{eff}}$</th>
<th>log $K_{oc}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil + 1% Slow Biochar</td>
<td>3.528</td>
<td>0.624</td>
<td>0.946</td>
<td>4.580</td>
<td>2.425</td>
</tr>
<tr>
<td>Soil +1% Fast Biochar</td>
<td>2.113</td>
<td>0.596</td>
<td>0.963</td>
<td>2.797</td>
<td>2.211</td>
</tr>
<tr>
<td>Soil with Biochar treatment</td>
<td>0.501</td>
<td>1.064</td>
<td>0.986</td>
<td>0.479</td>
<td>1.445</td>
</tr>
</tbody>
</table>

Table 3.10 Desorption isotherm fitted to the Freundlich equation for 17β-estradiol, Estrone, Progesterone

**Desorption Isotherms**

### 17-βestradiol (E2)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$K_f$</th>
<th>$N$</th>
<th>$R^2$</th>
<th>$K_{d_{eff}}$</th>
<th>log $K_{oc}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil + 1% Slow Biochar</td>
<td>325.987</td>
<td>0.852</td>
<td>0.954</td>
<td>361.279</td>
<td>4.322</td>
</tr>
<tr>
<td>Soil +1% Fast Biochar</td>
<td>228.929</td>
<td>0.848</td>
<td>0.931</td>
<td>254.382</td>
<td>4.169</td>
</tr>
<tr>
<td>Soil with Biochar treatment</td>
<td>21.622</td>
<td>1.445</td>
<td>0.982</td>
<td>15.880</td>
<td>2.965</td>
</tr>
</tbody>
</table>

### Estrone (E1)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$K_f$</th>
<th>$N$</th>
<th>$R^2$</th>
<th>$K_{d_{eff}}$</th>
<th>log $K_{oc}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil + 1% Slow Biochar</td>
<td>221.004</td>
<td>0.794</td>
<td>0.973</td>
<td>254.854</td>
<td>4.170</td>
</tr>
<tr>
<td>Soil +1% Fast Biochar</td>
<td>130.047</td>
<td>0.761</td>
<td>0.948</td>
<td>169.651</td>
<td>3.994</td>
</tr>
<tr>
<td>Soil with Biochar treatment</td>
<td>3.928</td>
<td>1.002</td>
<td>0.970</td>
<td>3.922</td>
<td>2.357</td>
</tr>
</tbody>
</table>

### Progesterone (P)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$K_f$</th>
<th>$N$</th>
<th>$R^2$</th>
<th>$K_{d_{eff}}$</th>
<th>log $K_{oc}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil + 1% Slow Biochar</td>
<td>69.759</td>
<td>0.459</td>
<td>0.998</td>
<td>101.484</td>
<td>3.770</td>
</tr>
<tr>
<td>Soil +1% Fast Biochar</td>
<td>26.638</td>
<td>0.416</td>
<td>0.949</td>
<td>39.936</td>
<td>3.365</td>
</tr>
<tr>
<td>Soil with Biochar treatment</td>
<td>13.804</td>
<td>0.832</td>
<td>0.982</td>
<td>15.512</td>
<td>2.955</td>
</tr>
</tbody>
</table>
Fig 3.1 Freundlich sorption isotherms of E2, E1 and P for soil and soil-biochar treatments

17β-estradiol Sorption

Estrone Sorption

Progesterone Sorption

log(Cs) (μg/g) vs log(Ce) (mg/L)
Fig 3.2 Sorption Isotherms of E2, E1 and P for soil and Soil-biochar treatment

17β-Estradiol Sorption

Sorbed Concentration ($C_s$) (μg/g) vs. Equilibrium Concentration ($C_{eq}$) (mg/L)

Estrone Sorption

Sorbed Concentration ($C_s$) (μg/g) vs. Equilibrium Concentration ($C_{eq}$) (mg/L)

Progesterone Sorption

Sorbed Concentration ($C_s$) (μg/g) vs. Equilibrium Concentration ($C_e$) (mg/L)
Fig 3.3 Freundlich desorption isotherms for E2, E1 and P for soil and soil-biochar treatments

**17β-estradiol Desorption**

**Estrone Desorption**

**Progesterone Desorption**
Fig 3.4 Sorption Isotherms of E2, E1 and P for soil and Soil-biochar treatment

17β-Estradiol Desorption

- Slow Biochar
- Fast Biochar
- Soil

Estrone Desorption

- Slow Biochar
- Fast Biochar
- Soil

Progesterone Desorption

- Slow Biochar
- Fast Biochar
- Soil
Fig 3.5 Scanning electron micrographs (SEMs) of (a) Fast pyrolysis biochar and (b) Slow pyrolysis biochar at 4 scales (500, 200, 100 and 50 μm)
3.7 Sorption-Desorption Behavior of the Natural Sex Hormones in the Presence of Biochar

3.7.1 Sorption isotherms

The sorption and desorption isotherms and Freundlich model for each of the three natural sex hormones (E2, E1 and P) in the soil and the soil-biochar treatments are given in Fig 3.1-3.4. The dimensionless Freundlich constant (n) for Progesterone, in 1% slow biochar treatment, with a value of 0.624 and 0.596 in 1% fast biochar, indicates high non-linearity of sorption isotherms in the presence of biochar. The sorption isotherms for estrogens (E2, E1) were slightly non-linear. The n value for E2 in the 1% slow biochar treatment was 0.942 and 0.949 in the 1% fast biochar treatment. The n values for E1 in the slow and fast biochar treatments were 0.919 and 0.942, respectively. There was a big difference between the non-linearity of the sorption isotherms of progesterone and estrogens, where the degree of nonlinearity of progesterone was much greater than that for both estrogen hormones. However, the non-linear sorption behavior of E2 and its metabolite, E1, was quite similar. For all three hormones, the n values, calculated in the soil treatments, were equal to one indicating the linearity of the sorption isotherms.

During the pyrolysis, at their specific glass transition temperature, the amorphous macromolecular materials transit from the glass phase to a rubbery phase (Xia and Pignatello, 2001). The carbonized fraction of biochar performs as the glassy domain function (i.e. the nonlinear sorption isotherms) and the non-carbonized fraction represents the rubbery domain function (i.e. linear sorption isotherms)(Zheng et al., 2010). The partition sorbent behavior of the rubbery state and the free voids are due to the rigidity of the glass state semi-permanent nano scale vadous zone of carbonized fraction of biochar which can be considered as adsorption sites with an internal high surface for the soil organic matter. The rubbery phase performance of the soft carbon domain and the similar glassy phase performance of the hard carbon domain, known as the two predominant domains in the heterogeneous sorbents (e.g. soil organic matters) with different sorption function, have been reported.

The sorption isotherms of E1 and progesterone demonstrated the S-type isotherms in both soil-biochar treatments. In S-type isotherms, the sorption affinity of the sorbent surface is
low and will increase at higher concentrations; after the saturation of the vadous zone, the slope becomes zero. The E2 sorption isotherms for the soil-biochar treatments were similar to L-type sorption Isotherms where the sorbent has a high sorption affinity at low concentrations and it decreases with an increase in the concentration. The sorption isotherms for all three hormones in soil treatments were C-type isotherms, which indicates the partitioning as the sorption mechanism by distribution of the compound in the solid-solution phase without any specific bonding between sorbent and sorption site (Sparks, 2003).

A significant difference (p<0.05) has been observed between the effective distribution coefficient ($K_{\text{d eff}}$) ($L/kg$) for each hormone undergoing the soil or soil-biochar treatment. The highest ($K_{\text{d eff}}$) value between the three hormones was found for E2 and the lowest ($K_{\text{d eff}}$) was reported for progesterone in both soil and soil-biochar treatments. A significant difference (P<0.05) between the ($K_{\text{d eff}}$) values can also be seen between the slow-pyrolysis and fast pyrolysis treatments for each hormone, where the slow pyrolysis biochar was shown to be more effective on sorption affinity of the hormones.

Several possible factors may explain the disparity between the sorption-desorption affinity of the two biochars for these three hormones. The differences between the physical characteristics of the two biochars contributed to the disparity of feedstock and the quality of the organic materials leads to differences in the specific surface area and cation exchange capacity, lower ash content and volatile matter, higher organic carbon content, different organic materials, particle size and the pyrolysis conditions. Peng et al. (2011) evaluated the effect of temperature and duration of pyrolysis on the chemical, physical, morphological and spectral properties of biochar. They reported that the aliphatic C groups decreased and the aromatic C increased during the charring, therefore, modifying the functional group in the structure of biochar.

For a better evaluation of the mobility of hormones in the soil and the effect of the biochar amendment, the soil/water-organic carbon partition coefficient ($K_{\text{oc}}$) was calculated for each hormone in both soil and soil-biochar treatments. The log ($K_{\text{oc}}$) for E2, E1 and progesterone were 1.77, 1.633, 1.445, respectively, in the soil treatment, which indicates the low sorption affinity of the hormones in sandy soil and high probability of
leaching from the soil. In the presence of the slow pyrolysis biochar, the log ($K_{oc}$) values for E2 were a 1.377 log unit larger, and in the fast biochar treatment the log ($K_{oc}$) values were 1.216 log units larger than the soil treatment. This can be considered as proof of the significant effect of biochar on increasing the sorption affinity of hormones. The difference between the soil and soil-biochar treatment for E1 was 1.423 log units and 1.253 log units for the slow and fast biochars, respectively. The sorption affinity of progesterone in the slow-pyrolysis biochar was 0.98 log units larger than for the soil, while it was 0.766 log units larger in the presence of the fast-pyrolysis biochar.

The effective distribution coefficient of E2 is increased 24 times more with the addition of the 1% slow pyrolysis biochar, as compared to the control (soil treatment); on the other hand, the 1% fast pyrolysis biochar was almost 16 times more effective. The effectiveness of biochar on the sorption behavior of E1 was slightly similar to its parent compound where the sorption affinity of this primary metabolite of estradiol was 26 and 18 times more in the presence of slow and fast biochars. However, the addition of 1% biochar to the sandy soil was significantly effective for progesterone sorption affinity. This effect was least among three hormones; this could be due to the differential chemical structures of progesterone and estrogens. The impact of the slow pyrolysis biochar on the sorption isotherms of the three hormones resulted in an increase of almost 1.5 times that of the fast pyrolysis biochar. The extremely small $K_{d}^{\text{eff}}$ for the soil treatment could be due to the coarse-particle structure of the soil where more than 93% of the texture is sand; however, it was observed that with the addition of biochar, the increase in sorption capacity of the sandy soil was, on average, 12 times greater. The values of the sorption isotherms (e.g., the $K_{d}$ effective values and the $K_{oc}$ values) of the estrogens in the soil treatment were similar to the values reported by Loffredo and Senesi (2002).

3.7.2 Desorption isotherms

Desorption isotherms for each of the three natural sex hormones (E2, E1 and P) in the soil and in the soil-biochar treatments have been calculated by fitting the desorption batch equilibrium data to the Freundlich model. The desorption of these hormones in the soil-biochar treatments were highly non-linear. The progesterone desorption isotherms were the most non-linear isotherms with $n$ values of 0.416 and 0459 in slow and fast biochar.
treatments respectively, where the n values for the estrogens ranged between 0.776 to 0.852 in both soil-biochar treatments. E1 demonstrated the smallest effective distribution coefficient at the desorption equilibrium ($K_d^{\text{eff}} = 3.922 \text{ L/kg}$) where the $K_d^{\text{eff}}$ for E2 and progesterone were quite similar in the sandy soil, indicating the higher possibility of E1 being desorbed and leached from the sandy soil as compared to progesterone and E2. A statistically significant difference ($P<0.05$) was observed between the soil-biochar and soil treatment in desorption of the hormones. The sorption resistance of slow biochar treatment for estradiol was highly stronger than for the other two hormones. A similar pattern was observed for the fast biochar treatment.

The rate of desorption in the soil-biochar treatment was significantly lower than the soil treatment; however, the ability of two biochars to keep the hormones sorbed to the soil-biochar media was not significantly different. Nevertheless, the slow biochar was more resistant to desorption of the hormones at lower concentrations. For a better comparison of the desorption behavior of each hormone, the percentage of desorption for each treatment was calculated. Desorption of hormones from the sandy soil treatment for four different sorbed concentrations ranged from 21% to 80%. This might be the result of the inability of sandy soil to keep the hormones sorbed to the soil matrix. Whereas, desorption of the three hormones in the soil-biochar treatment ranged from 0.05% to 13% of the initial equilibrium concentration. In the soil treatment, E1, with an average desorption rate of 72%, and E2, with a desorption rate of 58%, demonstrated a higher desorption potential than the 31% desorption of progesterone. Progesterone demonstrated the lowest desorption rate of 0.79% of the initial sorbed concentration in the slow biochar treatment and 2.5% desorption rate in the fast biochar treatment, whereas the desorption rate of both estrogens in both the slow and the fast biochar were similar, ranging from 7% to 12%.

Following the reported desorption hysteresis in soil-organic compound matrices, the sorption-desorption hysteresis was found in both soil and soil-biochar treatments as evidence of the non-linear sorption-desorption behavior of hormones. The non-ideal sorption behavior of hormones in the biochar treatments and the observed sorption-desorption hysteresis were compatible with results of the studies several studies (Beck et
al., 1993; Carmo et al., 2000; Sarmah et al., 2010; Satoh et al., 1995; Schlebaum et al., 1999). The sorption-desorption hysteresis and nonlinearity can be due to the different sorption functions of carbonaceous substances with high surface area (Xia and Pignatello, 2001) as the condensation sites in soil. Several factors, such as the chemical or biological transformation, non-equilibrium conditions, and high-energy bonding (Cox et al., 1997) have been considered as a potential explanation of the hysteresis phenomenon. Several studies, such as Cox et al. (1997) have attributed the non-ideal sorption behavior to the heterogeneity and structural properties of the soil organic matter (e.g., aromaticities), the total organic carbon, oxygen, and sulfur content of the soil media. Cox et al. (1997) quantified the dependency of sorption-desorption hysteresis on soil organic matter (SOM) and O/C(oxygen/carbon) atomic ratio. They also indicated the dependency of the sorption-desorption hysteresis on the residual aqueous-phase solution concentration, which could be due to the non-Fickian diffusion of the compound in the condensed glassy domain of soil organic matters.

3.8 Discussion

3.8.1 Effect of the biochar characteristics on the sorption and desorption mechanism of natural sex hormones in soil

The results of this study demonstrated a significantly higher sorption affinity and stronger desorption resistance for soil amended with 1% biochar, rather than the soil treatment, for the three hormones, thus, indicating a high retention capability of biochar as a soil amendment. This is similar to the results in recent studies (Chun et al., 2004; Lehmann et al., 2003; Sarmah et al., 2010; Spokas et al., 2009; Uchimiya et al., 2011; Yu et al., 2011; Zheng et al., 2010). For instance, Spokas et al. (2009) reported the enhanced sorption ability of Minnesota soil for herbicides such as atrazine and acetochlor where biochar was applied as soil amendment and increased the organic carbon in the soil. A statistically significant difference was observed between the retention ability of the two biochars. The retention capacity of different types of biochars is a function of several variables including the feedstock, pyrolysis conditions and temperature, carbon content and degree of aromatic condensation (Sarmah et al., 2010) which can cause higher sorption affinity and greater resistance to the desorption of organic contaminants in the
soil-biochar media. In this study, the slow-pyrolysis biochar demonstrated a stronger remediation capability than the fast-pyrolysis biochar. The lower volatile matter content in the slow pyrolysis biochar and therefore, the higher access to porous zones and a greater surface area (Allen-King et al., 2002; Sarmah et al., 2010) could be one reason for these observations. However, several studies (Enders et al., 2012; Peng et al., 2011; Sarmah et al., 2010) have reported that generally the higher pyrolysis temperature can lead to a higher surface area and pore volume characteristics of biochar. Our results demonstrated that the slow pyrolysis biochar is a stronger sorbent for the hormones. This observation can be due to the different feedstocks and production conditions. Also, Bornemann et al. (2007) reported that sometimes the higher temperature does not necessarily affect the higher surface area contribution or more porosity which can be due to the loss of active components in biochar. This leads to lower sorption characteristics including surface area and micro porosity (Sarmah et al., 2010).

### 3.8.2 Biochar sorption mechanism

Using scanning electron micrographs, shown in Figure 3.5, the rudimentary pore structure and the partial surface with tar-like deposits of each of the biochars, used in this investigation, could be seen. Generally, the structure of biochar consists of an aromatic C, aliphatic C, carboxyl and carbohydrate and volatile matter where pyrolysis and mineralization by microorganisms are the two major processes, which remove the volatile fraction of biochar including its aliphatic components with relatively lower C and higher O content (Peng et al., 2011). The preliminary phase of sorption of organic chemicals in the presence of biochar is a function of the gradual intra-particle diffusion mechanics where the characteristics of biochar, including the different particle size distribution, chemical decomposition and surface area, could influence the equilibrium sorption isotherm of organic compounds (Zheng et al., 2010). The carbonization of biochar is a fundamental function for the sorption behavior of biochar. Known as two major composition structures, carbonized and non-carbonized fractions are the main reason for the heterogeneous surface of biochar (Cao et al., 2009). The sorption capacity of biochar is dependent on several factors including the soil particle size distribution, the organic matter content, and the structural characteristics of biochar. Zheng et al. (2010) attributed
the sorption of the hydrophobic compounds from an aqueous solution to the highly hydrophobic surface of biochar.

3.9 Conclusion

The sorption-desorption behavior of the three manure-borne female sex hormones (17β-estradiol, estrone and progesterone) was evaluated in the presence of two different types of biochar (slow and fast pyrolysis), where both biochars demonstrated a high sorption affinity for hormones and a strong resistance in desorbing the hormones. It is proposed that the potency of biochar is important not only as an organic soil amendment for improving the soil quality and crop production but also as a novel remediation technique for better sustainable conservation of soil and water resources. Based on the detailed analysis of the biochar characteristics, the aromatic carbon backbone in the porous, amorphous structure of biochar appears to influence the sorption behavior of organic contaminants. Nevertheless, the sorption capacity of biochars, produced from different feedstocks, would be different and, from this point of view, a more detailed investigation is required to evaluate the influence of the different feed stocks and production conditions on the characteristics of biochar. The sorption and desorption isotherms indicated that, in both sorption and desorption batch equilibrium experiments, slow pyrolysis biochar showed a better support for our hypothesis of the remediation capacity of biochar to reduce hormonal pollution in agricultural soil, amended with animal manure. From an environmental and agricultural perspective, the outcome of this study can be seen as proof of the need for further field-scale applications of biochar as a novel, clean and feasible remediation technique. However, detailed, long-term studies are required to investigate the competitive sorption ability of biochar in the simultaneous presence of different chemicals in the soil media.
PREFACE TO CHAPTER 4

Poultry and liquid swine manure are the most widely applied organic fertilizers to agricultural soils in North America. They are used to boost the soil quality as a multi-purpose agronomic practice; however, from the environmental and health safety perspective, they are major sources of bioactive levels of natural steroidal sex hormones, including 17β-estradiol, estrone, testosterone and progesterone.

In this Chapter, the environmental fate and transport of the manure-borne female sex hormone, progesterone, was investigated under two different treatments, with and without biochar, over a 45-day period, using a field-scale lysimeter study where both poultry and swine manure were applied to the topsoil as an organic fertilizer.

To date, this is the first field study highlighting the influence of the retention potential of slow-pyrolysis biochar on the environmental behavior, fate and transport of manure-borne progesterone in soil media. This research will help us understand the role of biochar in improving the water quality and can be potentially used in agricultural fields to mitigate the concerns of manure-borne hormonal pollution.

Research papers based on the chapter:

S.Alizadeh, F.Gobbi, S.O.Prasher  Effect of Slow Pyrolysis Biochar on the Fate and Transport of Manure-Borne Progesterone in Soil  (under preparation)
Chapter 4
Effect of Slow Pyrolysis Biochar on the Fate and Transport of Manure-Borne Progesterone in Soil

4.1 Abstract

Due to lack of knowledge regarding the environmental and aquatic fate and transport of progesterone and simultaneously inadequate knowledge to address the remediation of steroid hormones in the soil matrix and aquatic media, a detailed field-scale lysimeter study was conducted to investigate the fate and transport of manure-borne progesterone. The main objective of this study was to develop an economically feasible remediation technique in order to reduce the environmental and biological consequences of hormonal pollution. The spatial–temporal stratification of progesterone was monitored over a 45-days period where two different types of manures (swine and poultry) was applied to the topsoil of lysimeters under two treatments (i.e. soil vs. soil amended with 1% slow pyrolysis biochar). A significant difference (p<0.05) was observed between the spatial-temporal stratification of progesterone in soil under the two treatments, at four sampling depths including the surface, 15, 35 and 65 cm over 45 days. The significant lower mass of progesterone quantified at different depths of the soil profile and the measured concentration of progesterone in water samples over time in the lysimeters, amended with 1% slow pyrolysis biochar, potentially confirmed the hypothesis of this study, which confirmed the retention capability of biochar as a soil-amendment for reducing manure-borne hormonal pollution in soil and water.

Keyword. Steroid sex hormones, progesterone, lysimeter, pyrolysis

4.2 Introduction

Over the last decade, advances in analytical techniques has made possible the detection of biologically active, organic micro pollutants known as emerging contaminants in soil and water (Pignatello et al., 2010). The endocrine disrupting properties and the high, chronic toxicity causing adverse long-term biological, development and health issues
(e.g. carcinogenicity, mutagenicity or teratogenicity) of these organic contaminants, at concentrations as low as ng/L (Choi et al., 2004), has meant that the occurrence, fate and transport of these emerging contaminants have become of particular concern in environmental studies. Environmental exposure to these toxic chemicals potentially modulates the normal functions of the endocrine system by altering or interfering with the metabolism and biosynthesis of endogenous hormones (Colucci et al., 2001; Sonnenschein and Soto, 1998). The primary concerns of endocrine disrupting compounds are natural and synthetic steroid hormones which have been identified as the major source of several cases of sexual and reproductive abnormalities observed in the aquatic environment. Androgens, estrogens, and progestagens are classified as the three main categories of steroid sex hormones. Female sex hormones play an influential role in the biological system. Nevertheless, it is the elevated concentrations of these sex hormones in environmental matrices that have been distinguished as one of the potential sources of female endocrine disorders such as polycystic ovary syndrome (PCOS) and breast cancer, numerous cases of prostate and testicular cancers (Pruden et al., 2006) as well as several incidences of inter sexual disorders and development and reproduction abnormalities in the aquatic environment (Guillette Jr et al., 1995; Tyler et al., 1998; Waring and Harris, 2005).

The main focus of the environmental studies is on the fate and transport of the free and conjugated forms of the female sex hormones including estrogens (17β-estradiol (E2), its primary metabolite, estrone(E1)) and progesterone due to their high biotransformation potential and environmental stability.

Known as early precursors in the formation of other steroid hormones, progesterone is produced by both sexes with significantly higher concentration in females. As its direct reproductive role, it is responsible for preparing the uterus for the fertilized egg and for maintaining pregnancy; meanwhile, it also plays an important role on nervous system, influencing mating and parental care behavior (Bevacqua et al., 2011). Synthesized from cholesterol, the chemical structure of progesterone is similar to other steroids; it is composed of three cyclohexanes and a cyclopentane ring with two ketone functional groups and two methyl groups which affect its behavior (Bevacqua et al., 2011; Kreinberg, 2012). The behavior of progesterone is pH-independent due to a lack of
proton-accepting functional groups in its structure (Kreinberg, 2012). Progesterone is relatively hydrophobic with log $K_{ow}$=3.87 (Neale et al., 2009) with a reported low bioavailability which means it will be easily excreted by livestock and, therefore, it can enter the environment. However, the common, natural exposure of steroid hormones is the excretion by vertebrates in the intensive industrial agriculture and expanding anthropogenic actives including concentrated animal feeding operations and agricultural and land management practices including the application of biosolids and animal manure and wastewater plant (WTPs) discharges. These have been frequently identified as a major source of environmental availability of female sex hormones. From the agricultural perspective, poultry and liquid swine manure are the most widely applied organic fertilizers to agricultural soils in North America (Zitnick et al., 2011). They are used to boost the soil quality as a multi-purpose agronomic practice; however, from the environmental and health safety perspective, these manures are major sources of bioactive levels of natural steroidal sex hormones, including $17\beta$-estradiol (E2), estrone (E1), testosterone and progesterone.

Recently, several studies investigated the environmental occurrence and fate of estrogens due to their ability to affect the reproductive biology of aquatic vertebrate species at extremely low concentrations while being detected in concentrations above their lowest observation effect level (LOEL), 10 ng/L (Shore and Shemesh, 2003) in environmental matrices. The environmental behavior and fate of progesterone is less well-known. The frequent contamination of surface water and ground water by manure-borne steroid hormones by surface runoff from agricultural fields receiving animal manure is reported in several studies (Shore and Shemesh, 2003; Soto et al., 2004; Warnock et al., 2007). Frequently detected concentrations of estrogens in the of soil under a corn field receiving manure was reported by Allen-King et al. (2002). A limited number of studies have reported on the occurrence of biologically active concentrations of progesterone in the surface soil and runoff from agricultural fields treated with manure. Lange et al. (2002b) indicated the excretion of androgens and progestins at rates comparable to, or greater than, rates for estrogens. The incidence of these steroids in watersheds with animal agriculture has been reported (Barel-Cohen et al., 2006; Kolodziej et al., 2004; Soto et al.,
Kolpin et al. (2002) reported the contamination of 4.3% of streams in the United States with average progesterone concentrations of 0.11 μg/L and a maximum concentration of 0.199 μg/L. Mansell et al. (2011) investigated the occurrence and pathways of six steroid hormones, including progesterone, in beef cattle feedlot runoff after simulated rainfall where they detected biologically active concentrations of progesterone in soil and runoff samples.

Bartelt-Hunt et al. (2012) investigated the occurrence of 16 endogenous and synthetic steroid hormones and metabolites in runoff from beef cattle feedlots and in manure and soil collected from feedlot surfaces where they detected progesterone in both soil and manure samples and runoff samples from feedlots with an average concentration of 59.5 ng/L and a maximum concentration of 570 ng/L. However, Schwarzenberger et al. (1996) indicated the key role of rapid metabolic transformation of progesterone influencing its environmental concentration by rapidly decreasing of its concentration, the potential stability of progesterone is expected following the previous detection of the compound in surface water resources (Kolpin et al., 2002). Kolodziej and Sedlak (2007) investigated the contribution of rangeland grazing areas to steroids in surface waters where the measured concentrations and estimates from stream discharge used to estimate the loading of steroids to surface waters where progesterone was detected in 5% of streams within the rangeland of roaming beef cattle, with a maximum concentration of 27 ng/L (Kreinberg, 2012).

Although over the last decade, several detailed investigations of environmental pathways and ecotoxicology of high-toxicity-at-low-concentrations of estrogens in soil and water media have been conducted, there is a lack of knowledge regarding the environmental and aquatic fate and transport of progesterone. Due to inadequate knowledge to address the remediation of steroid hormones in the soil matrix and aquatic media, there is a pressing need to conduct detailed studies with the objective of developing economically feasible remediation techniques in order to reduce the environmental and biological consequences of hormonal pollution.
4.2.1 Biochar

The thermo-chemical conversion of biomass and biological residues (e.g. wood, poultry litter, crop residues, etc.) in the absence of oxygen (or partially combusted in the presence of a limited oxygen supply) is known as pyrolysis which leads to environment-friendly and sustainable energy production and waste management. Carbonizing the organic material in biomass through pyrolysis results in the production of energy-rich and valuable end products (Meng et al., 2013) including bio-oil, combustible gases and a fine-grained carbonaceous residue, known as biochar. Based on the pyrolysis conditions including different heating rates, various types of biochars with specific structural and physio-chemical properties are produced. Slow pyrolysis biochar is the final by-product of pyrolysis at relatively low temperatures (300-500 °C) with a long heating time and a heating rate less than 10 °K/min whereas fast pyrolysis takes place over a short time at a high temperature (700-900 °C) with a heating rate of more than 1000 °K/min (Mohan et al., 2006).

The amorphous structure, containing nano-scale condensed aromatic rings with a crystalline structure and therefore the strong sorption affinity, high specific surface area and resistance to bio-decomposition of biochar offer an approach to engineer the natural process in order to fulfill an environmental remediation of inorganic contaminants (e.g. heavy metals) and hydrophobic organic contaminants (Beesley et al., 2011). The strong sorption affinity of carbon rich amendments, such as black carbon, activated carbons and biochar for poly-aromatic hydrocarbons and other categories of organic contaminants may make it possible to use them as a novel bio-remediation technique. Besides its ability to improve soil quality and fertility as an ameliorant (Farrell et al., 2013), due to their highly adsorptive properties, the in-situ application of biochar as a soil amendment has been proposed as a multi-purpose and financially-feasible approach to reduce the bioavailability and bioaccumulation of organic contaminants. Despite the environmental application of black carbon or activated carbon to remediate the organic contaminants, their limited sorption ability caused by the blockage of their porous carbon backbone structure (Winsley 2007) may need to be activated before they are incorporated into the soil media. However, biochar consists of a porous, carbonaceous structure with an
extremely high surface area, which provides strong sorption sites in contaminated soil media.

Improving the soil physio-chemical properties, including the increased cation exchange capacity (CEC), provides more nutrient availability and better fertility, increased pH levels, and higher water holding capacity water infiltration. Recent studies (Enders et al., 2012; Novak et al., 2009; Oguntunde et al., 2004; Van Zwieten et al., 2010; Warnock et al., 2007) reported improved soil physio-chemical properties as a result of biochar amendment. Several investigations (Rondon et al., 2005; Spokas et al., 2009; Troy et al., 2013) demonstrated the significant role of biochar as a soil amendment or resulted in the reduction of greenhouse gas emission. The retention ability of biochar on the mobility, bioavailability and toxicity of heavy metals and polycyclic aromatic hydrocarbons (PAHs) is reported by Beesley et al. (2011). The reduced pore-water concentration of heavy metals and therefore, the effectiveness of biochar on the mobility of copper, lead and plant uptake of heavy metals was assessed by Karami et al. (2011). Yu et al. (2011) investigated the sorption capacity of biochar on different types of pesticides including triazine and acetamiprid. The only investigation to evaluate the sorption capacity of biochar for estrogens was conducted by (Sarmah et al., 2010). Several laboratory scale studies investigated the remediation of organic contaminant bioavailability by the application of activated carbon and biochar (Cho et al., 2009; Herman and Mills, 2003; Millward et al., 2005; Sun and Ghosh, 2008; Zimmerman et al., 2004); however, due to inadequate knowledge regarding environmental remediation behavior and sorption capacity of biochar under field conditions, more detailed temporal investigations of in situ field-scale retention ability of biochar are needed.

To best of author’s knowledge, this is the first field scale investigation assessing the retention potency of slow pyrolysis biochar as a topsoil amendment while simultaneously monitoring the fate and transport of the naturally manure-borne progesterone in sandy soil over a 45-day period where poultry and liquid swine manure were applied as fertilizer to outdoor lysimeters irrigated with different levels of simulated rainfall. We hypothesize that we will address the question of the utility of using a novel bio-remediation technique through the application of biochar for the reduction of manure-
borne hormonal pollution in soil and water to provide better sustainable soil and water conservation.

4.3 Methodology

4.3.1 Experimental Setup

Twelve PVC lysimeters were used to evaluate the influence of biochar on the fate, transport and retention of the manure-borne female sex hormone, progesterone, in soil and water media; six lysimeters received poultry manure and remaining six lysimeters received liquid swine manure. The twelve lysimeters were located at the Macdonald Farm on the Macdonald campus of McGill University in Ste-Anne-de-Bellevue, Quebec. Each lysimeter was 45 cm diameter × 100 cm height. To collect the leachate after irrigation, each lysimeter was sealed at the bottom with a 60cm×60cm PVC sheet and a 0.5 cm diameter drainage pipe was installed in the bottom of each one. The lysimeters were packed with layers of sandy soil with a bulk density of 1350 kg/m³. Four 10 mm in diameter holes were drilled in each lysimeter at 0.15, 0.35 and 0.65 m below the soil surface in order to collect soil samples at different depths. To prevent any plant uptake, plant residues were removed from the soil surface and crops were not planted. The lysimeters were sheltered by a canopy to prevent the entry of natural rainfall and the moisture content of each lysimeter soil was brought to field capacity. Figure 4.1 shows the layout of the lysimeter.

4.3.2 Soil characteristics

The lysimeters were kept under natural field conditions. They were packed with sandy soil (with 92.2 % sand content) of the Ste-Amable complex, Ferro-Humic podzol, provided by the farm on the Macdonald campus of McGill University, Ste-Anne-De-Bellevue, Quebec in 1993. No hormone residue was found in the initial soil samples; however, after the last application of manure to the lysimeters in 2007 (Fan, 2009), soil samples were collected from the lysimeters to determine the initial concentrations of the estrogen hormones.
The organic matter content was determined by the loss on ignition. The physical characteristics of the soil are provided in Table 4.1. The hydraulic parameters of unsaturated sandy soil were estimated using the Rosetta program (Schaap et al., 1998) where the saturated water content (Vol%) was 43.4% (cm$^3$/cm$^3$), residual water content (Vol%)= 5.19% (cm$^3$/cm$^3$) and saturated hydraulic conductivity of the soil was estimated as 26.15 (cm/day).

Table 4.1 Physical characteristics of the soil (Adopted from Fan (2009))

<table>
<thead>
<tr>
<th>Soil texture</th>
<th>Physical Properties</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand</td>
<td>Saturated hydraulic conductivity Ksat (m/d)</td>
<td>3.68</td>
<td></td>
</tr>
<tr>
<td>Silt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clay</td>
<td>Organic matter content</td>
<td>2.97%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td></td>
<td>5.5</td>
</tr>
</tbody>
</table>

*S.D. denotes standard deviation

1 Cation exchange capacity

Fig 4.1 The Schematic diagram of the lysimeter
4.3.3 Weather Data

During the period of the experiment, the maximum, minimum and average air temperatures were 21.2, 9.8 and 15.6 °C, respectively, and the average relative humidity was 66%. All the meteorological data were collected from the Ste-Anne de Bellevue station of Environment Canada.

4.3.4 Source of poultry and swine manure

Poultry manure was provided by the Donald McQueen Shaver Poultry Complex, located at the Howard Webster Centre for Teaching and Research in Animal and poultry Science, Macdonald campus of McGill University, Quebec, Canada. The Poultry complex contains 3200 layers and 1600 broilers. Based on the documents provided by R. Howard Webster Centre, the poultry’s diet did not contain any growth promoting, or other synthetic hormones or antibiotics. The detailed poultry diet plan included corn, wheat, soybean, rye, barley, phosphorus, vitamins and calcium.

Liquid swine manure was provided by the Macdonald campus swine complex located at the R. Howard Webster Centre for Teaching and Research in Animal and Poultry Science, Macdonald campus of McGill University, Quebec, Canada. The Swine Complex is a farrow to finish operation with a capacity of 48 sows. Swine received several clinical veterinary treatments beyond their weaning phase, including PG600 and regumate, to control and induce the estrus, lutalyse and oxytocin for inducing and speeding up farrowing and Duplocillin LA but the swine were not given any synthetic hormones or antibiotics.

The detailed analysis of both the swine and the poultry manure was conducted in February 2012 by AgroEnviro Lab, La Pocatière, Quebec, Canada; the data is presented in Table 4.2. The poultry and swine manure were collected one day before initiating the field study and before the first irrigation. Sterilized 2L Pyrex glasses were used for the manure collection and for transferring to the lysimeter site. The manure extraction and hormone-content analysis of manure was performed prior to the field study.
Table 4.2 Nutrient analysis of liquid swine and poultry manure

<table>
<thead>
<tr>
<th></th>
<th>Swine manure</th>
<th>Poultry manure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (M.S %)</td>
<td>0.1</td>
<td>26.1</td>
</tr>
<tr>
<td>Organic matter (M.O. %)</td>
<td>0.6</td>
<td>17</td>
</tr>
<tr>
<td>Density (t/m³)</td>
<td>0.977</td>
<td>0.622</td>
</tr>
<tr>
<td>C/N ratio</td>
<td>2.8</td>
<td>5.8</td>
</tr>
<tr>
<td>Total Nitrogen (kg/t)</td>
<td>1.1</td>
<td>14.7</td>
</tr>
<tr>
<td>Ammonia Nitrogen (N-NH₄)</td>
<td>0.45</td>
<td>5.25</td>
</tr>
<tr>
<td>Phosphors (P₂O₅)(kg/t)</td>
<td>0.4</td>
<td>11.7</td>
</tr>
<tr>
<td>Potassium (K₂O) (kg/t)</td>
<td>1.3</td>
<td>8.4</td>
</tr>
<tr>
<td>Calcium (Ca) (kg/t)</td>
<td>0.3</td>
<td>22.1</td>
</tr>
<tr>
<td>Magnesium (kg/t)</td>
<td>0.1</td>
<td>1.5</td>
</tr>
</tbody>
</table>

4.3.5 Manure application

Based on the yield adjustment and nutrient requirement, an estimated rate of 170 m³/ha was chosen for the spring liquid swine manure application and 2.7 L of liquid manure was applied directly to the soil surface of each lysimeters. The application rate of poultry manure was calculated for silage corn as a crop with the typical yield of 50 ton/ha and nitrogen requirement for given yield of 20 kg (N)/ha. 400g of poultry manure was incorporated into the first 10 cm topsoil of each lysimeter with the application rate of 25 ton/ha, assuming spring as the application time.

4.3.6 Manure extraction and hormonal content analysis of poultry manure

A modified extraction procedure was performed following the methods used by Andaluri et al. (2012). Sterilized 50 mL Pyrex glass centrifuge tubes were used to weigh 4g (wet weight) of poultry manure samples. Liquid extraction was conducted by adding twice 4 mL methanol and twice 4 mL acetone solvents. At each step, after adding the solvent, the samples were placed on a reciprocating shaker with a speed of 250 rpm for 30 minutes and the contents of the tubes were centrifuged at 4000 rpm for 20 minutes. The transparent supernatant of each tube was transferred to another clean, 50 ml tube. After collecting the liquids, the final extract was filtered by a 0.45-μm syringe filter and brought to a final volume of 1,000 mL in high purity deionized water. The extraction was processed by a solid phase extraction procedure for the samples as described in section 4.13.2. The final extracts were filtered by 0.22 μm sterile filters and transferred into the
amber HPLC vials and analyzed as described. 1.92±0.68 (μg/Kg) of progesterone was quantified in the analyzed poultry manure samples.

4.3.7 Manure extraction and hormonal content analysis of liquid swine manure

In order to quantify the progesterone content in swine manure in the laboratory, a modified extraction procedure was performed following the methods used by Andaluri et al. (2012) and Thompson et al. (2009). Three replicates of 5 ml of swine manure samples were measured in the sterilized 50 ml Pyrex glass centrifuge tubes and liquid extraction was conducted by adding twice 4 ml methanol and twice 4 ml acetone solvents. At each step, after adding the solvent, the samples were placed on a reciprocating shaker with a speed of 250 rpm for 30 minutes and the contents of the tubes were centrifuged at 4000 rpm for 20 minutes. The transparent supernatant of each tube was transferred to another clean, labeled 50 ml tube. After collecting all liquids, the final extract was filtered by a 0.45-μm syringe filter and brought to a final volume of 1,000 ml in high purity deionized water. The extraction was processed by a solid phase extraction procedure for the samples as described in section 4.13.2. The final extracts were filtered by 0.22 μm sterile filters and transferred into the amber HPLC vials and analyzed as described. 0.57±0.15 (μg/Kg) of progesterone was quantified in the analyzed swine manure samples.

4.3.8 Biochar characteristics

BlueLeaf biochar was provided by BlueLeaf Inc, Drummondville, Quebec, Canada. BlueLeaf biochar is produced from the slow pyrolysis of soft wood at 450 °C. The soil control lab of Control Laboratories Inc., Watsonville, California, USA provided the detailed physical, chemical and elemental characterization of the slow pyrolysis biochar. The detailed physical and chemical characteristics have reported in Chapter 3, section 3.3.3.

4.3.9 Biochar application rate

Each soil-biochar treatment contained 1%(w/w) of biochar, based on the integration of the biochar at the first 10 cm of the topsoil with an application rate of 10t/ha (Sarmah et al., 2010).
4.3.10 Rainfall simulation

To simulate natural rainfall, the lysimeters were irrigated four times on days 0, 15, 30 and 45 after the application of the manures. To simulate the spring application of manure and to imitate the worst-case scenario, the highest total amount of rainfall in May in a fifty-year period from 1962-2012 was determined to be 174.3 mm. This amount was divided into three equal irrigations with a measured quantity of 57.8 mm for the month of May. The fourth irrigation was assumed to be equal to the other three irrigations. The irrigations consisted of a quantified amount of water being distributed on the soil surface of each lysimeter in four-hour segments at the just-ponding rate and at the center of the soil surface to prevent flooding of the surface and the loss of water from the open edges around the lysimeter’s body. The water application rate of 14.45 mm/h was based on the rainfall intensity distribution curve for Sainte-Anne-de-Bellevue, representing a one-in-eighteen year storm.

4.3.11 Soil and leachate sample collection method

After applying the manure, eleven sampling dates, including one day following each of the four irrigations, were assigned for collecting the soil samples. These were collected from the lysimeters’ topsoil as well as at the depths of 15, 35 and 65 cm, at day 0,1,3,7,15,16,23,30,31,45 and 46. One day after each irrigation (day 1, 16, 31 and 46) were added to the sampling dates in order to determine the effect of different levels of soil moisture content on the behavior of each hormones in soil media over time. Sterilized straws were used to collect composite soil samples at each sampling depth through four sampling ports in the side of the lysimeter. The samples collected from common treatments and depths were mixed and a 5 g subsample was obtained for other procedures. The soil samples were stored in sterilized sealed bags and frozen at -24 °C until the extraction and analysis could be performed. The moisture content of each soil sample was determined before they were frozen. Amber water bottles were acid washed and sterilized before they were used to collect the leachate from the drainage pipes at the bottom of each lysimeter. During irrigation, approximately 7 liters of leachate was collected from each lysimeter. Once, the collected leachates were homogenized, a one-liter subsample was obtained for subsequent extraction and analysis. Water samples were
collected at day 0, 15, 30 and 45 and transported to the lab, extracted and analyzed on the same day.

4.3.12 Mass balance calculations

The concentration of progesterone (C-mg/g) measured with analytical technique in the laboratory, moisture content of the soil samples (θ)(mass water/mass dry soil, mg/g) through different soil layers (h, cm), the bulk density of the soil (ρ, g/cm³) and the soil layer surface area in the each lysimeter (m²), were used to calculate the mass of hormones recovered in soil samples using the equation provided by Xia and Pignatello (2001).

\[
\text{Mass of Hormone} = [C \cdot θ \cdot h \cdot ρ \cdot a]
\]

The summation of the recovered amount of progesterone from leachate extraction and soil samples were used to determine the total recovered mass of hormone from each lysimeter. This provides an indication of the initial hormone mass introduced to the soil by the manure application minus any unrecovered hormones or other losses due to chemical or microbial degradation.

\[
\text{hormone (initial)} \ (\text{mg}) = 1590.4 \ \rho \ [C_{(0-5)} \cdot θ_{(0-5)} \cdot h_{(0-5)} + C_{(5-15)} \cdot θ_{(5-15)} \cdot h_{(5-15)} + C_{(15-35)} \cdot θ_{(15-35)} \cdot h_{(15-35)} + C_{(35-65)} \cdot θ_{(35-65)} \cdot h_{(35-65)}] + [C_{\text{leach}} \cdot V_{\text{leach}}] + E_{(\text{lost})}
\]

Where C_{\text{leach}} is presenting the hormone concentration in the leachate samples (mg L⁻¹), and V_{\text{leach}} is volume of leachates (L). The subscripts 0-5, 5-15, 15-35, and 35-65 represent the soil depth increments and 1590.4 is the calculated cross section surface area of each lysimeter.

4.3.13 Extraction of hormones from soil and leachate samples

All soil samples were collected at 4 depths from each lysimeter. They were subsampled and kept in an oven for 24 h set at 105 °C in order to measure the soil moisture content at different depths through time. The extraction of hormones from the soil samples was performed based on the modified method used by Xuan et al. (2008). Soil extraction was conducted in triplicate for the collected soil samples. 50 ml polyethylene centrifuge tubes
were used to weigh 5 g of soil after defrosting at room temperature. After the addition of five grams of anhydrous sodium sulfate (Na$_2$SO$_4$) and 10 ml acetone to each tube containing soil samples, the materials were mixed thoroughly for 1 minute using a Vortex mixer and this was followed by 30 min of vibrantly shaking with 250 rpm speed on a reciprocating shaker. The soil mixture content of tubes were centrifuged at 4000 rpm for 20 minutes and the transparent supernatant of each tube was transferred to another clean 50 mL tube. In the second step of the extraction, another 10 ml of acetone was added to all tubes and the extraction procedure was repeated. By transferring the supernatant collected after the second step, the tubes, containing the aliquots, were centrifuged at 4000 rpm for 20 minutes and the resultant supernatants were transferred to 50 mL pyrex glass centrifuge tubes and dried completely under N$_2$ stream. They were reconstituted with 1 ml of 50/50 (v/v) acetonitrile-water solution and kept in the sonicator for 15 minutes. The final extracts were filtered by 0.22 μm sterile filters and transferred into the amber HPLC vials and analyzed as described.

After collection, all the lysimeter leachates were transferred to the laboratory and extracted and analyzed on the same day. The leachate extraction was conducted as described in Stafiej et al. (2007) using solid phase extraction with Oasis HLB extraction cartridges(200 mg/cc)(Oasis Co.Ltd,NY). Briefly, 1 liter of each leachate sample was filtered through a 45 mm filter (advantec, Japan) to separate the suspended soil particles and other materials. 35% concentrated hydrochloric acid was used to acidify the water samples to pH 2. After conditioning the cartridges with 5 mL of methanol followed by 5 mL of high-purity de-ionized water, the acidified samples were passed through the cartridges with a flow rate of 20 mL/min. After eluting the compound with 10 ml of acetonitrile, the extract was dried under the nitrogen stream. The dried samples were dissolved in 1 mL of 50/50 (v/v) acetonitrile-water solution and were kept in the sonicator for 15 minutes. The final extracts were filtered by 0.22 μm sterile filters and transferred into the amber HPLC vials and analyzed as described.

4.3.14 Analysis of soil and leachate extracts

High performance liquid chromatography (HPLC) analysis was carried out to detect and quantify the progesterone, and the other two estrogen hormone standards. All the analysis
and concentration quantification of these hormones in the soil extracts and aliquots were performed by a quaternary pump LC system from Agilent 1100 series technologies (Germany) equipped with a diode array-ultraviolet detector.

4.3.15 Separation, identification and quantification of E2 and E1

The specific concentration of progesterone standard solution was prepared in 50/50 (V/V) purified Milli-Q water and HPLC-grade acetonitrile. The detection and concentration tracing of progesterone was carried out through a Zorbax Eclipse Plus C18 column (150×4.6 mm) with particle size of 5 μm (Agilent, Santa Clara, CA). The best detector response for the identification of progesterone was at 240 nm with the retention of 7.5 min. The mobile phase used in this process was a volumetric mixture of ratio of 40% of purified Milli-Q water and 60% HPLC-grade acetonitrile, with a flow rate of 1 mL/min with the injection volume of 100 μL for isocratic analysis. Prior to each analysis, the mobile phase was filtered by 0.45 μm membrane filters and degassed. The column temperature was kept constant at 25 °C during all analysis. To identify the retention time and the peak geometry of the compound, the injection of pure standards of hormone, with a different range of concentrations (5-0.001 mg L\(^{-1}\)), was performed. By using the external standard peak areas from chromatogram, at specific concentrations, a seven-point calibration curve equation for each compound was based on the linear regression in Excel (Microsoft Office Software). Furthermore, in order to determine the concentration of progesterone in the field samples, the peak’s areas, which were obtained from the each sample chromatogram, were compared with the hormone standards, peak’s areas and the concentrations were quantified by the standard calibration curves. The limit of detection for the progesterone was 0.001 mg L\(^{-1}\).

4.4 Statistical Analysis

The hypothesis of the significant effect of the remediation ability of biochar to reduce the hormonal pollution in soil and water was statistically analyzed by the spatial and temporal repeated measures model using PROG GLM in SAS v.9.2 (SAS Institute Inc, 2010)
4.5 Results

4.5.1 Progesterone distribution in soil and water samples

The spatial-temporal distribution concentration of progesterone in soil under the two treatments, soil and soil-biochar and the two different manures, at four sampling depths including the surface, 15, 35 and 65 cm over 46 days are presented in Figs 4.2 and 4.3. The distribution and partitioning patterns of manure-borne hormones would be controlled by their physicochemical properties as sorbates and the physical and structural properties of soil as a sorption site since the effects of site-specific environmental conditions should not be neglected (Ying and Kookana, 2009).

4.5.2 Spatial-temporal movement of progesterone in soil receiving poultry manure

The statistical analysis of the quantified concentration of progesterone in soil samples demonstrated a significant differences (p<0.05) between the soil and soil-biochar treatments with respect to both time and depth. A descending trend over time was observed for progesterone concentrations, found at depth 0 (lysimeter’s top soil), under both treatments. The significant concentration of progesterone was found at the soil surface after the first irrigation at day 0 in the lysimeters receiving 1% slow pyrolysis biochar. One day after the application of poultry manure and the first irrigation, 98.40±6.85 (μg/kg) of progesterone was measured at the soil surface in the presence of biochar; however, only 51.34±2.26 (μg/kg) of progesterone was quantified under the soil treatment. The two times lower concentration of progesterone under soil treatment even after the first irrigation could be considered a good indication of the rapid leaching and movement of progesterone in the sandy soil.

The higher concentration of progesterone (36.70±3.93 μg/kg) was found in topsoil at the first two days after the manure application, the major distribution of hormone was found at depth 15 and 35 cm under the soil treatments through time. 28.72±1.26 (μg/kg) and 14.58±2.04 (μg/kg) of hormone was found at depths of 15 and 35 cm at day 0, respectively, whereas the concentration of progesterone at the same depth under the biochar was measured as 14.10±2.04 (μg/kg) and 1.69±0.51 (μg/kg). The significantly lower analyzed concentrations of progesterone at depths of 15 cm and 35 cm in the
lysimeters under the biochar treatment even after the first irrigation potentially demonstrates the effective sorption ability of slow pyrolysis biochar for the retention of hormones in soil. The concentration distribution of progesterone over depth and time under biochar treatment was significantly different from soil treatment (P<0.05). After the application of poultry manure, the initial concentration of progesterone of manure in the soil surface was rapidly decreased more than 30% after the first irrigation in lysimeters without the 1% biochar amendment. More than 70% of progesterone was lost after 3 days in topsoil and only 23% of the initial concentration was found in the soil surface after 7 days. 4.79±1.16 (μg/kg) of progesterone was detected on day 15 and the measured concentration of 0.89±0.14 (μg/kg) on day 16, one day after the second irrigation, indicating the availability of only 1.8% of the primary hormone’s concentration on the soil surface. Less than 0.1% of progesterone (0.07 μg/kg) was detected on day 23 and the concentration of progesterone was below the detection limit.

More than 62% of the initial progesterone was detected in the topsoil after 3 days in the lysimeters with the biochar amendment. 43% of the initial manure-borne hormone was quantified after 7 days. 23.99±0.64 (μg/kg) of progesterone was measured on day 15 before the second irrigation which was found to be 5 times higher than the measured concentration under the soil treatment. Almost 10% of the initial manure-derived progesterone was available in the soil-biochar matrix in the soil surface. Even 2% of the initial progesterone (1.78±0.056 μg/kg) was detected in the lysimeters receiving biochar treatments even after the third irrigation on day 30. No concentration of progesterone was quantified between day 30 and after the fourth irrigation on day 45, under both treatments. The analyzed concentrations of progesterone in soil samples at lower depths demonstrated a major spatial distribution of the hormone in the initial 15 days at depth 15 cm in the range of 4.37-31.20 (μg/kg) and at a depth of 35 cm in the range of 6.83-22.87 (μg/kg) in lysimeters under soil treatment. The concentration of progesterone followed an ascending-descending trend over the first 15 days at both lower depths with the peak concentration of 31.20±2.43 μg/kg at day 1 at 15 cm depth and 22.87±0.77 μg/kg at depth 35 cm on day 15.
The lower depth’s distribution of progesterone in the lysimeters receiving biochar followed an ascending-descending trend over time but with significantly lower concentrations when compared to the soil treatment data. The maximum concentration of progesterone (15.52±0.31 μg/kg) was detected on day 1 at depth 15 cm which was half of the detected concentration at the same depth under soil treatment. The highest detected progesterone content at depth 30 cm was quantified 4.91±0.79 μg/kg indicating three times lower concentration as compared to similar reported value under soil treatment. No progesterone was detected at both lower depths under biochar treatment after the second irrigation on day 15. No progesterone was found in the soil samples collected from the depth of 65 cm in the presence of biochar in the entire 45 day period. Nevertheless, a detectable concentration of progesterone was found at depth 65 cm under both treatments in the range of 1.036-2.745 μg/kg after the second irrigation, indicating the potential of manure-borne sex hormones to leach downward in the soil. Increasing concentrations followed by a descending trend in the spatial distribution of concentrations at lower depths was observed over time, which is considered as an indication of the highly significant interaction between time and depth while a significant difference was found in analyzed concentrations of progesterone in soil with respect to both time and depth through statistical analysis.

**4.5.3 Spatial-temporal movement of progesterone in soil receiving swine manure**

The progesterone distribution in the lysimeters receiving liquid swine manure was found to be similar to the hormone distribution observed in the lysimeters receiving poultry manure. A descending trend over time was observed for progesterone concentrations at depth 0 under both treatments. Major portions of the initial manure-borne progesterone were distributed in the topsoil under biochar treatment. A significant difference (p<0.05) between the soil and soil-biochar treatments was found, based on both time and depth statistical analysis of the quantified concentration of progesterone in soil samples. 48.37±3.25 (μg/kg) and 68.72±1.2 (μg/kg) of progesterone was quantified in the soil surface under biochar and soil treatments, respectively, one day after the application of swine manure. The major distribution of hormone was found at depth 15 and 35 cm under the soil treatment through time whereas significantly lower analyzed concentrations of
progesterone were observed in the lysimeters under biochar treatment. The concentration of progesterone was measured 15.68±3.31 (μg/kg) and 5.34±0.85 (μg/kg) at depth of 15 and 35 cm at day 0 respectively under the biochar treatment whereas 27.61±1.36 (μg/kg) and 14.53±0.88 (μg/kg) of hormone was found at the same depths respectively under soil treatment. The temporal distribution of progesterone under the two treatments was significantly different (P<0.05) through depth and time. The initial amount of progesterone introduced to the soil surface by the application of swine manure rapidly decreased by almost 50% after the first irrigation in the lysimeters without the 1% biochar amendment and only 21% of preliminary progesterone was detectable on day 3. Only 5.83±0.49 (μg/kg) of progesterone representing 10% of the initial concentration of progesterone was quantified at day 7 and one day after the second irrigation, only 2% of progesterone was available in the soil surface. Only 0.1 (μg/kg) of progesterone was found in the collected soil sample from the soil surface on day 23 and no progesterone was detected after the next two irrigations.

The analysis of soil samples collected from the lysimeters treated with biochar demonstrated the presence of more than 65% and 48% of initial progesterone in the topsoil after 3 and 7 days, respectively. The biochar-soil mixture was able to keep almost 38% of the initial hormone content in the topsoil after the second irrigation. 8.558±1.10 μg/kg of progesterone was detected on day 23 in the lysimeters under biochar treatment which was 8 fold greater than the concentration found in the lysimeters under soil treatment. Even though no progesterone was detected under soil treatment in the soil surface after 30 day, a traceable concentration of hormone (2.15±0.26 μg/kg) was found in the upper part of soil profile on day 30 under the biochar treatment. No concentration of progesterone was quantified between day 31 and after the fourth irrigation on day 45 under both treatments. Based on the observed spatial-temporal stratification of progesterone in the soil profile, the major concentration of progesterone was distributed in the initial 16 days at depth 15 cm in the range of 1.97-36.67 (μg/kg) and at a depth of 35 cm in the range of 2.57-19.58 (μg/kg) in the lysimeters under soil treatment. The temporal distribution of progesterone at both lower depths followed an ascending-descending trend over the first 15 days, whereas the highest hormone content was detected on day 1 with the peak concentrations of 36.67±1.34 μg/kg at day 1 and at 15 cm
depth and 19.57±0.94 μg/kg at depth 35 cm on day 7. The temporal hormone distribution under biochar treatment followed a descending pattern at depth 15 cm with significantly lower progesterone concentrations as compared to soil treatment. No hormone was detected after the second irrigation at depth 15 cm in the lysimeters receiving 1% biochar. The maximum concentration of progesterone (10.1 ± 0.71 μg/kg) was detected on day 1 at depth 15 cm which was three times lower than detected concentration at the same depth under soil treatment. The temporal stratification of progesterone at depth 35 cm demonstrated 6.90±0.10 μg/kg of progesterone at day 7 as the highest concentration detected in the presence of 1% biochar in topsoil; this was almost 9 times lower than the similar reported value under soil treatment. The hormone content of lysimeters with biochar decreased to less than 0.44±0.10 μg/kg one day after the second irrigation and no progesterone was detected after day 16 until the end of study on day 46. The spatial distribution of hormone over time demonstrated that biochar was able to keep the manure-derived hormone in the top layers of the soil profile since no progesterone was found in the soil samples collected from the depth 65 cm in the presence of biochar in the entire 45 days; however, a trace concentration of progesterone found at depth 65 cm under soil treatment treatments in the range of 0.163-1.63 μg/kg between the second and third irrigations. No hormone was detected after day 30.

4.5.4 Temporal presence of progesterone in leachate samples after the application of poultry manure

The manure-borne progesterone content of the leachate samples, collected after each irrigation on days 0, 15, 30 and 45, was quantified using HPLC analysis. The average concentration of progesterone in the leachate samples under the two treatments over time is provided in Fig 4. Significant detectable trace concentrations of progesterone (μg/L) were found in the leachate samples; however, the quantified progesterone content of water samples was relatively lower than the hormone concentrations measured in soil samples. The quantified concentration of progesterone in the leachate samples collected from lysimeters under biochar treatment was significantly lower (P<0.05) than the determined progesterone content in the leachate samples under soil treatment. The highest concentration of hormone over the entire 45 days and after four irrigations was measured after the first irrigation on day 0 with the initial concentration of 72.92±5.21
(μg/L) in the leachate samples under the soil treatment. The highest progesterone concentration under the biochar treatment was also found on the first day; however, it was 42% of detected progesterone under soil treatment. In treatments, the hormone’s concentrations measured in leachate samples were decreased over time; for example, 40.95±0.41 (μg/L) and 18.36±3.82 (μg/L) of progesterone was detected on day 15 and 30, respectively. The concentration of progesterone was below the detection limit after the fourth irrigation on day 45 under the soil treatment. The quantified concentration of progesterone from lysimeters receiving biochar was 2 times lower than the other treatment at day 15 and on day 30, after the third irrigation, only 1.90±0.44 (μg/L) of progesterone was measured in water samples collected under biochar treatment. This represented only 5% of the detected value under soil treatment. No hormone was detected after the fourth irrigation on day 45.

4.5.5 Temporal presence of progesterone in leachate samples after the application of swine manure

The temporal stratification of progesterone in analyzed leachate samples receiving liquid swine manure was found to be similar to the results from samples collected under poultry manure treatment. For both treatments, a significant detectable trace concentration of progesterone (μg/L) was found in the leachate samples; however, the quantified progesterone content of the water samples was relatively lower than the hormone concentrations measured in soil samples. The quantified concentration of progesterone in the leachate samples collected from lysimeters under biochar treatment was found to be significantly lower (P<0.05) than the determined progesterone content in the leachate samples under soil treatment. Based on the temporal distribution of the hormonal content of the leachate samples, demonstrated in Fig 4.4, the highest concentration of progesterone (117.49±13.08 μg/L) was quantified after the first irrigation on day 0 under the soil treatment. The quantity of progesterone was twice the maximum progesterone (46.23±13.08 μg/L) found in the leachate samples under the biochar treatment.

A descending pattern was found in the stratification of progesterone over time in both treatments. The hormone content quantified in leachate samples was reduced by more than 65% after 15 days and after the second irrigation. 9.94±1.84 (μg/L) of the
progesterone was detected on day 30; however, the concentration of progesterone was lower than the detection limit after the fourth irrigation on day 45 under the soil treatment and almost 3 times lower than the progesterone in the water samples collected on day 15 under the biochar treatment. Only 1.75±0.53 (μg/L) of the hormone was detected at day 30 and none, after the fourth irrigation on day 45.

4.5.6 Mass balance

The quantified concentration of manure-borne progesterone was used to conduct the mass balance of the hormone in the soil and water samples for all eleven sampling days. The residual hormone content in different depths of the soil profile and collected leachate samples through time for the two treatments are presented in Table 4.6 and 4.7. Initially, 767.12±3.25 μg and 1515.30±2.94 μg of progesterone were introduced to the topsoil of the lysimeters by applying poultry and swine manure, respectively.

4.5.6.1 Mass balance for progesterone from poultry manure

After the first irrigation, more than 58% of the preliminary progesterone dissipated in the soil profile rapidly in the lysimeters under soil treatment. Based on the spatial-temporal stratification of the progesterone mass balance, a major concentration of progesterone was found at a depth of 15-45 cm in the soil profile in the initial days of the experiments under soil treatment. Under the biochar treatment, the dominant hormone content in the soil profile was found in the first 10 cm where the biochar was incorporated. The mass of progesterone which was found to be maximum on the day 0 at the lower depths under the soil treatment, decreased over time; after 30 days no significant mass of progesterone was detected. The mass distribution of progesterone in the presence of biochar was significantly different, whereas generally the mass of progesterone under biochar treatment was 4 times lower than similar values under soil treatment. No hormones were detected between 45-75 cm of the soil profile in the lysimeters with 1% biochar. There was a significant mass of progesterone ranging from 10.12-15.37 μg quantified under the soil treatment.
Fig 4.2 Concentration of Progesterone in the soil and soil-biochar treatments at four sampling depth of lysimeter receiving Poultry manure (log scale used for Y axis plotting)
Fig 4.3 Concentration of Progesterone in the soil and soil-biochar treatments at four sampling depths of lysimeter receiving swine manure (log scale used for Y axis plotting)
On day 0, the preliminary progesterone mass in the lysimeters under soil treatment was 362.57±0.95 μg and two days after the application of manure, only 35% of the total initial progesterone was recovered. The mass balance analysis of progesterone content quantified under the biochar treatment demonstrated the ability of biochar to retain more than 68% of the initial progesterone derived from poultry manure one day after the application of manure whereas the initial progesterone mass content under the biochar treatment was calculated to be about 524.81 ±0.64 μg. The comparison of the mass distribution of progesterone at four depths of the soil profile demonstrated the ability of biochar to retain hormones in the soil surface and a strong desorption resistance which decreased the leaching rate of hormones toward subsurface water. For instance, 15.51±0.62 μg of progesterone was detected in the first 5 cm of soil under the soil treatment on day 3, whereas the P mass under biochar treatment measured 146.45±1.25 μg. After the second irrigation, the progesterone content of the soil surface in the
lysimeters receiving 1% biochar was 4 times greater than was detected in the lysimeters under soil treatment. No progesterone was detected in the different soil layers under soil treatment on day 30, after the application of the rainfall simulation. However, 1.85±0.54 μg of hormones was still detectable in the soil surface under biochar treatment. The analyzed mass of progesterone found in the in the leachate samples collected from the drainage outlet of the lysimeters receiving 1% biochar was half of the hormone mass detected in the samples from lysimeters without remediation treatment after the first two irrigations. The mass of progesterone under the biochar treatment was not statistically significant after the third irrigation on day 30; nevertheless, a trace mass of progesterone was still detectable in the water samples collected under the soil treatment. No progesterone mass was found under both treatments after the last irrigation in day 45.

4.5.6.2 Mass balance for progesterone from swine manure

The spatial-temporal stratification of the progesterone mass balance in the lysimeters receiving liquid swine manure was similar to progesterone mass profile for the poultry manure. After the application of swine manure, a dominant portion of the progesterone mass was found at a depth of 15-45 cm in the soil profile in the preliminary days of the experiments under the soil treatment; however, the major mass of progesterone was concentrated in first 10 cm of soil profile in the presence of biochar. The highest mass of progesterone was detected in topsoil on day 0 under both treatments. Nevertheless, progesterone was found in the biochar-soil mixture at levels almost three times greater than the soil treatment content. The mass of progesterone decreased rapidly at both the surface and at lower layers of the soil profile over the first 30 days. A significant difference was observed between the two treatments. Only 20% of the initial manure borne progesterone (310.31±0.34 μg) was quantified in the topsoil of the lysimeters under soil treatment one day after the application of the liquid manure. In the case of biochar, more than 35% of the initial progesterone was detected in the soil surface, but biochar demonstrated a stronger retention ability to hold the manure-borne progesterone in the case of poultry manure. This observed difference may be a case of the different physical properties of the manures, including the liquid/solid phase manures. No hormones were detected between 45-75 cm of the soil profile in the lysimeters with 1% biochar;
however, a significant mass of progesterone ranging from 1.19-12.25 μg was quantified under the soil treatment.

Two days after the application of manure, only 40% progesterone, which was measured on day 0, was recovered; almost 63% of the preliminary progesterone from day 0 was still available on the soil surface in the biochar media. After 7 days, 26.01±0.75 μg of progesterone was detected in the first 5 cm of soil under the biochar treatment whereas the P mass under soil treatment was 2.25 ± 0.56 μg in the topsoil. The spatial mass contribution of progesterone for three lower depths of the soil column under biochar treatment was found to be generally half of the mass distribution of progesterone in lysimeters under soil treatment. No hormone was detected in 45 to 70 cm of soil profile in lysimeters receiving biochar. Even after the second irrigation on day 15, no progesterone was measured from 15 cm to 70 cm of the soil profile in the same lysimeters. The analysis of leachates collected after each irrigation under both treatments demonstrated a significantly lower mass of progesterone in the samples collected from drainage outlet of the lysimeters receiving 1% biochar. No progesterone was found under both treatments after applying the last irrigation on day 45.

These results indicate a better potential for remediation by biochar as a soil amendment where biochar provides a heterogeneous sorption site consisting of pre-existing meso- and micropore cavities with different pore sizes and pore surfaces. This results in the heterogeneity of nonspecific sorption-site energies where van der Waals forces play the dominant role as the solute-sorbent interactions (Xing et al., 1996). The association of hormones with dissolved or colloidal fractions of soil and/or manure can be considered as another possible explanation for hormone persistence and greater than expected mobility in soil (Cox et al., 1997). Johnson and Amy (1995) reported that humic substances can act as mobile, or flowing, solid phase and therefore, increase the mobility of sorbed contaminants by enhanced desorption of polycyclic aromatic hydrocarbons (PAH) in low organic carbon soil. However, humic substances have a sorption ability similar to the stagnant solid phase; they have the mobility potential similar to, or greater than, the mobile aqueous phase (McGechan and Lewis, 2002). The preferential flow integrated or mixed through convective–dispersive processes in water-saturated pores is
another influencing factor governing the transport of manure-borne progesterone in the soil profile (Fan et al., 2007). Another parameter controlling the spatial and temporal stratification of progesterone is the texture and physical properties of the soil. The high sand content of the soil (more than 92%) would potentially lead to less sorption and more progesterone would be dissolved in the aqueous phase as a result, causing more leaching of the hormones to lower depths. Less available oxygen in the lower part of the soil profile and therefore, less biological activities (Fan et al., 2007) can be considered as a possible reason for the longer persistence of hormones in the lower depths.

4.5.7 Degradation kinetics of progesterone under the field condition

The first-order decay model (Johnson, 2001) was found to be the governing model determining the degradation kinetics of manure-borne progesterone under soil and biochar treatments, receiving poultry and liquid swine manure. Equation (3) shows the detailed components of the degradation model:

\[
\frac{dC_t}{dt} = -kt 
\]

Integrating Eq. (3) gives

\[
C_t = C_0 e^{-kt} 
\]

Where \(C_t\) is the residual hormone concentration at time (t), \(C_o\) is the initial concentration, and \(k\) is the degradation rate constant. The half-life of progesterone was calculated based on the plotted natural logarithm of mass/initial mass of hormone versus time and slope of the linear graph representing the degradation rate constant.

<table>
<thead>
<tr>
<th>Poultry manure</th>
<th>Regression slope (K)</th>
<th>Coefficient of determination (r^2)</th>
<th>Half-life (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>0.2552</td>
<td>0.92</td>
<td>3</td>
</tr>
<tr>
<td>Biochar</td>
<td>0.1097</td>
<td>0.92</td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Swine manure</th>
<th>Regression slope (K)</th>
<th>Coefficient of determination (r^2)</th>
<th>Half-life (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
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<td>0.91</td>
<td>3</td>
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<tr>
<td>Biochar</td>
<td>0.1019</td>
<td>0.93</td>
<td>7</td>
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The coefficient of determination ($r^2$) of each degradation equation under both treatments demonstrates that the first-order rate degradation model can explain the degradation of manure-borne female sex hormones in soil under field conditions. A significant difference ($p<0.05$) was found between the degradation rate constant of progesterone under biochar and soil treatment in lysimeters receiving poultry manure. The degradation rate constant of hormone under biochar treatment was calculated as $0.0046 \, h^{-1}$ whereas the same parameter was $0.01606 \, h^{-1}$ under soil treatment. Similar results were observed in the lysimeters receiving swine manure, where the degradation rate factor in the presence of biochar was $0.00945 \, h^{-1}$ and $0.00425 \, h^{-1}$ under soil treatment. However, the determined half-life of progesterone was significantly different under both treatments; a similar degradation pattern was observed for both hormones for lysimeters receiving poultry and swine manure. However, the half-life of progesterone was determined as 6 days under biochar treatment. It was observed that progesterone degraded two times faster in lysimetres under soil treatment with a half-life of 3 days in the case of poultry manure. Similarly, in the lysimeters amended with swine manure, half of the progesterone concentration degraded in 7 days in the presence of biochars; however, the half-life for the hormone was 3 days under soil treatment.
Table 4. Mass balance profile of progesterone (μg) in the lysimeter soil profile and leachate samples over the 45 day period (poultry manure)

<table>
<thead>
<tr>
<th>Soil profile (cm) Day</th>
<th>Soil treatment</th>
<th>0-5</th>
<th>5-15</th>
<th>15-45</th>
<th>45-70</th>
<th>Cum. leachate</th>
<th>Total</th>
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<td>**</td>
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<table>
<thead>
<tr>
<th>Soil profile (cm) Day</th>
<th>Soil-Biochar treatment</th>
<th>0-5</th>
<th>5-15</th>
<th>15-45</th>
<th>45-70</th>
<th>Cum. leachate</th>
<th>Total</th>
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<td></td>
</tr>
</tbody>
</table>

*Mass of progesterone (μg) in total collected volume of leachate samples after each irrigation

**Below the detection limit of progesterone in analytical process

*a Standard deviation
Table 4.5 Mass balance profile of progesterone (µg) in the lysimeter soil profile and leachate samples over the 45 day period (swine manure)

<table>
<thead>
<tr>
<th>Soil profile (cm)</th>
<th>Soil treatment</th>
<th>0-5</th>
<th>5-15</th>
<th>15-45</th>
<th>45-70</th>
<th>Cum. leachate</th>
<th>Total</th>
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<tr>
<td>Day</td>
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<tr>
<td>0</td>
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<td>117.36</td>
<td>98.36</td>
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*Mass of progesterone (µg) in total collected volume of leachate samples after each irrigation

**Below the detection limit of progesterone in analytical process

a Standard deviation
The degradation of manure-borne hormones has been investigated through several laboratory studies in the presence of various organic matrices, including animal wastes and biosolids under aerobic conditions. It was proven that the major degradation of hormones in soil is caused by microbial degradation influenced by temperature and the pH of the media (Khanal et al., 2006). Detailed information regarding the biotic and abiotic degradation of estrogens in soil media is available, for instead the reported rapid microbial degradation of 17β-estradiol with a half-life of 0.17 day in non-sterilized soil by Xuan et al. (2008); however only limited estimated regarding the degradation of progesterone has been presented (Mansell et al., 2011). Through our investigation, the degradation rate factor of manure-borne progesterone excreted from two difference sources of manure (poultry and manure) was calculated where the degradation rate constant of poultry manure-borne progesterone was 0.01606 h⁻¹ and 0.00425 h⁻¹ in the presence of swine manure. Also, the significant influence of the redox conditions (aerobic vs. anaerobic) on the degradation of hormones should be considered. (Holthaus et al., 2002; Jürgens et al., 2002; Ying and Kookana, 2009). Kjær et al. (2007) found that the incorporation of manure containing sex hormones in the topsoil would result in soil conditions potentially remaining anoxic for a longer period of time and therefore would retard the degradation of hormones with a greater chance of bioavailability.

4.5.8 Role of biochar

The hypothesis of this investigation proposes the remediation potential of slow pyrolysis biochar for reducing manure-borne hormonal pollution; it was confirmed by the significant differences found between the spatial and temporal distribution of progesterone concentration under biochar and soil treatments in the presence of both poultry and liquid swine manure. Significant sorption affinity and strong desorption resistance of biochar are considered as the two key parameters governing its sorption-desorption behavior and therefore, the aqueous concentration of progesterone, resulting environmental availability and degradation of the hormone. The result of this study is compatible with recent studies reporting the positive sorption ability of different types of biochars for the reduction of various emerging contaminants, including antibiotics, herbicides, pesticides and heavy metals (Chun et al., 2004; Lehmann et al., 2003; Sarmah
et al., 2010; Spokas et al., 2009; Uchimiya et al., 2011; Yu et al., 2011; Zheng et al., 2010).

Fig 4.5 Natural logarithm of mass/initial mass ($m/m_0$) of progesterone versus time under two treatments in the presence of both manure

4.6 Conclusion

The environmental fate and transport of the manure-borne female sex hormone, progesterone, was investigated under two different treatments, with and without biochar, over a 45-day period using a field-scale lysimeter study where both poultry and swine manure were applied to the topsoil as an organic fertilizer. The main objective of the study was to evaluate the retention ability of slow pyrolysis biochar as a soil-amendment to reduce the manure-borne hormonal pollution in soil and water under field conditions.
A significant difference (p<0.05) was observed between the temporal–spatial stratification of progesterone in soil under the two treatments, soil and soil-biochar, at four sampling depths including the surface, 15, 35 and 65 cm over 46 days in the case of both manures. The significant lower mass of progesterone quantified at different depths of the soil profile and the measured concentration of progesterone in water samples over time in the lysimeters amended with 1% slow pyrolysis biochar, potentially confirmed the hypothesis of this study, which posed the highly effective retention capability of biochar as a soil-amendment for reducing manure-borne hormonal pollution in soil and water.

These results indicate the significant potency of biochar, not only as an organic soil amendment for improving soil quality and crop production, but also as a novel remediation technique to regulate the environmental bioavailability and degradation of manure-borne sex hormones and therefore, reduce hormonal pollution in soil and water to reach a sustainable conservation of water resources. The aromatic carbon backbone in the porous, amorphous structure of biochar may explain the higher sorption affinity and stronger desorption resistance of biochar, which influences the sorption behavior of organic contaminants. The sorption capacity and retention potential of biochar is a function of the pyrolysis and production conditions and the preliminary feedstock of the biocar. Since there is inadequate knowledge and limited data to address the remediation ability of biochar for regulating the environmental emerging contaminants pollution, more detailed temporal field-scale studies are required. To date, this is the first field study highlighting the influence of the retention potential of slow-pyrolysis biochar on the environmental behavior, fate and transport of manure-borne progesterone in soil media. This study has proposed the amendment of biochar as a novel, clean and feasible remediation technique from the environmental and agricultural perspective. However, detailed, long term, time–scale studies are required to investigate the competitive sorption ability of biochar in the simultaneous presence of different chemicals in the soil media.
PREFACE TO CHAPTER 5

Estrogens are the dominant naturally manure-borne female sex hormones. In the previous chapter, the significance of biochar was determined for reducing the manure borne progesterone contamination in soil and water samples in lysimeters. In this chapter, the environmental fate and transport of the manure-borne estrogens was investigated under two different treatments, with and without biochar, over a 45-day period using a field-scale lysimeter study where poultry manure was applied to the topsoil as an organic fertilizer.

To date, this is the first field study highlighting the influence of the retention potential of slow-pyrolysis biochar on the environmental behavior, fate and transport of manure-borne estrogens in soil media. This research will help us understand the role of biochar in improving the water quality and can be potentially used in agricultural fields to mitigate the concerns of manure-borne hormonal pollution.

Research papers based on the chapter:

Chapter 5
Evaluation of Slow Pyrolysis Biochar in Reducing Estrogen-Based Female Sex Hormones in Poultry Manure

5.1 Abstract

The present study’s primary objective was to monitor the fate and transport of the two manure-borne steroidal sex hormones (17β-estradiol and estrone) in sandy soil over a 45-day period. The study’s secondary objective was to evaluate the remediation potential of slow-pyrolysis biochar topsoil amendments as a novel bio-remediation technique. The significant differences (p<0.05) were found in the analyzed concentrations of estradiol and estrone in soil samples between the soil treatment and soil-biochar treatment with respect to both time and depth. The comparison of mass balance data for each depth of soil profile and leachate samples demonstrates the significant retention ability of biochar for sorption of hormones in the soil surface and decreasing the leaching rate of hormones toward the subsurface water. The results of this study have highlighted the significant retention ability of biochar for reducing the manure-borne hormonal pollution in agricultural soil media. From an environmental and agricultural perspective biochar can be proposed as a novel, clean and feasible remediation technique. However, detailed, long term time-scale studies are required to investigate the competitive sorption ability of biochar in the simultaneous presence of different chemicals in the soil media.

Keyword. Estrogens, biochar, lysimeter, leachate, soil

5.2 Introduction

As a result of increasing anthropogenic activities such as concentrated animal feeding operations, considerable amounts of animal wastes are being generated; these animal wastes are applied to the land as an organic soil-amendment, which is now a routine way of disposing of animal wastes. However, this waste management technique has become a major gateway for environmental exposure to a new class of toxic and high-risk organic contaminants known as emerging contaminants. These micro pollutants are poorly documented in terms of their toxicological and biological risks for the environmental, health and safety issues (Schriks et al., 2010). The emerging contaminants are known to
have endocrine disrupting properties and may stimulate high, chronic toxicity and adverse, long-term health issues (e.g. carcinogenicity, mutagenicity or teratogenicity) at concentrations as low as ng/L (Choi et al., 2004). Therefore, they have been classified as endocrine disrupting compounds (EDC) with the ability to modulate the functions of the endocrine system by mimicking, counteracting, altering or interfering with the metabolism and biosynthesis of endogenous hormones (Colucci et al., 2001; Sonnenschein and Soto, 1998). Sexual and reproductive abnormalities and intersexuality in the aquatic environment, caused by interference with the normal endocrine system and physiological functions, indicate a strong connection between environmental occurrence and the presence of natural and synthetic steroidal sex hormones.

Both primary female and male sex hormones are synthesized from cholesterol with common a cyclopentan-o perhydrophenanthrene ring in their chemical structure, which is produced and released by the adrenal cortex, ovary and placenta in vertebrates and humans (Lehmann and Rondon, 2006). The major concerns among steroid hormones are the free and conjugated forms of the female sex hormones including, 17β-estradiol (E2), its primary metabolite, estrone (E1) and progesterone. Known for their high biotransformation potential and environmental stability, these female sex hormones have been detected in concentrations above their lowest observation effect level (LOEL), 10 ng/L (Shore and Shemesh, 2003), in environmental matrices while causing physiological disorder at lower concentration than other steroid hormones.

The predicted-no-effect-concentration (PNEC) of 1.0 ng L⁻¹ of E2 and 3-5 ng L⁻¹ of E1 were reported by Van Zwieten et al. (2010), while Novak et al. (2009) detected concentration levels as low as 3.3 ng L⁻¹ E1 and 14 ng L⁻¹ E2 as the lowest observable effect level (LOEL) affecting vitellogenin production in fish species (juvenile female rainbow trout). The feminization of male fish or the masculinization of female fish (Soto et al., 2004; Vajda et al., 2011), the reproductive biology alteration of wild fathead minnows (Pimephales promelas) and rainbow trout at aquatic E2 concentrations as low as 1-10 ng L⁻¹ and 25-50 ng L⁻¹ of E1 (Fang et al., 2003; Young and Borch, 2009) or the intersexuality in fish exposed downstream of wastewater treatment plants
and drainage effluents (Blazer et al., 2007; Woodling et al., 2006) represent several biological hormone disruption incidences in aquatic media.

Although the excretion by vertebrates is a common natural exposure to steroid hormones, the land application of animal waste and biosolids (e.g. sewage sludge) generated by concentrated animal feeding operations (CAFO’s) and wastewater treatment plant (WTPs) discharges are the major sources of environmental exposure to steroid sex hormones in soil and water media. Therefore, the fate, transport, pathways and ecotoxicology of potentially high-risk, natural steroid sex hormones have been the dominant goal of recent environmental studies. From the agricultural perspective, the land application of animal waste is considered a beneficial waste management strategy to boost soil fertility and quality and yet, the land application of animal manure has been identified as a high-risk source of potential environmental hormonal pollution. The soil biogeochemical processes including sorption and desorption, physiochemical transformation (Chowdhury et al., 2010; Zhang et al., 2007) and biodegradation into other metabolites or mineralization highly influence the environmental persistence and dissipation of steroid hormones in the soil matrix. The physicochemical properties of steroid sex hormones as sorbates, the physical and structural properties of soil as a sorption site and site-specific environmental conditions (Ying and Kookana, 2009) influence the distribution and partitioning patterns of estrogens. Known as nonvolatile, slightly hydrophobic compounds that do not ionize at normal environmental pH (Hanselman et al., 2003), estrogens demonstrated relatively high sorption coefficients, which is consistent with the low aqueous solubility of these hormones (Lee et al., 2003).

In spite of the reported short half-lives and fast dissipation under aerobic condition, the high sorption affinity of these hormones in topsoil (Casey et al., 2005; Das et al., 2004; Lee et al., 2003), several studies (Shore and Shemesh, 2003; Soto et al., 2004; Warnock et al., 2007) have indicated the hormonal contamination of water resources by surface runoff from agricultural fields receiving animal manure. These hormones have been found in surface water and ground water (Cox et al., 1997; Kolodziej et al., 2004; Orlando et al., 2004; Pignatello et al., 2006). Frequently detected concentrations of estrogens in the porous zone under a corn field receiving manure was reported by Allen-
King et al. (2002). The potential leaching of manure-borne sex hormones via macrospore flow, particularly high concentrations of E2, three months after the application of manure, was demonstrated in the investigation of transport of estrogenic hormones from manure-treated structured loamy soil to tile drainage system by Kjær et al. (2007). They detected both E2 and E1 in drainage water at concentrations exceeding the LOEL up to 11 months after the application of manure. Herman and Mills (2003) report the frequent detection of estrogens in the vadose zone beneath a manure-fertilized corn leachate. In the investigation conducted by Finlay-Moore et al. (2000), runoff concentrations of 100-2500 ng L$^{-1}$ of E2 was found after the application of poultry manure. In the last decade, the occurrence, pathways and ecotoxicology of high-toxicity-at-low-concentration steroid hormones in soil and water media have become one of the hotspots in environmental studies (Combalbert and Hernandez-Raquet, 2010; Hansen et al., 2011; Jenkins et al., 2008; Sangsupan et al., 2006; Steiner, 2009; Ternes et al., 2002). Inadequate knowledge and paucity of studies addressing the remediation of these sex hormones in the soil matrix and aquatic media have resulted in an important subject for environmental research area.

### 5.2.1 Biochar

Recently, the application of organic soil-amendments, derived from biological matter, has been proposed as a feasible remediation technique in order to reduce the risk of pollutant transfer to the aquatic environment and to receptor organisms (Beesley et al., 2011). The minimum pretreatment requirement means they can be applied directly to the soil and provides a convenient way to dispose of organic waste. They are able to bind pollutants and reduce their bioavailability while promoting plant growth and stimulating ecological restoration (Vangronsveld et al., 2009). The in-situ application of carbon-rich amendments to contaminated soils has been deployed as a financially-feasible approach to engineer the natural process in order to fulfill an environmental remediation requisite (Beesley et al., 2011). The dominant potential of carbonaceous fractions of these organic amendments has led to the deliberate introduction of clean types of these fractions (e.g. activated carbon, biochar) into sediments, to reduce the bioavailability of organic contaminants (Zimmerman et al., 2004). Two orders of magnitude higher sorption of organic contaminants to soils and sediments have been reported on the basis of sorption
to natural organic matter due to the presence of these additional carbonaceous fractions (Cornelissen et al., 2005). The retention ability of these materials is reported in several environmental remediation investigations (Beesley et al., 2011; Brändli et al., 2008).

The incomplete combustion of organic materials (e.g. coal or coconut shells), followed by activation to increase the surface area (Brändli et al., 2008), will lead to the production of strongly sorbing carbonaceous charcoal materials known as activated carbon. However, biochars are also produced by combustion processes; their feedstocks would be derived from biological residues such as wood, poultry litter, crop residues, etc., which are commonly activated or further treated before application to soils (Beesley et al., 2011). Specifically, the thermo-chemical decomposition of biomass and biological residues (e.g. wood, poultry litter, crop residues, etc.) in the absence of oxygen (or partially combusted in the presence of a limited oxygen supply) is known as pyrolysis and leads to the production of bio-oil, combustible gases and a fine-grained carbonaceous residue, named biochar. Based on the pyrolysis conditions, including the heating rate (i.e. fast, intermediate or slow pyrolysis), the properties of the biochar would be different. The slow pyrolysis would take place at relatively low temperatures (300-500 °C) with a long heating time and a heating rate less than 10 °K/min with high yields of solids, i.e., biochar, whereas fast pyrolysis takes place over a short time at a high temperature (700-900 °C) with a heating rate of more than 1000 °K/min (Mohan et al., 2006). The amorphous structure, containing nano-scale condensed aromatic rings with a crystalline structure and the presence of both polar and non-polar surface sites of biochar offer a strong sorption affinity for inorganic contaminants (e.g. heavy metals) and hydrophobic organic contaminants due to its high specific surface area and resistance to biodecomposition.

Recent studies have documented the significant role of biochar as a soil amendment for reducing greenhouse gases, nitrogen oxide (N₂O) and methane (CH₄) emissions (Rondon et al., 2005; Spokas et al., 2009; Troy et al., 2013). The positive effect and retention capacity of biochar on the mobility, bioavailability and toxicity of heavy metals and polycyclic aromatic hydrocarbons (PAHs) is reported by Beesley et al. (2011). Karami et al. (2011) assessed the impact of biochar on the mobility of copper and lead and plat
uptake of heavy metals, where biochar was effective in reducing the pore-water concentration of heavy metals. The sorption capacity of biochar on different types of pesticides, including triazine and acetamiprid, was investigated by several studies (Chun et al., 2004; Smernik, 2009; Yu et al., 2010; Zheng et al., 2010). However, while there are several laboratory and field-scale studies examining the remediation of organic contaminant bioavailability by the application of activated carbon (Cho et al., 2009; Herman and Mills, 2003; Millward et al., 2005; Sun and Ghosh, 2008; Zimmerman et al., 2004), there is lack inadequate information and few investigations of in situ field-scale applications of biochar. The only investigation to evaluate the sorption capacity of biochar for estrogens at the laboratory scale was conducted by Sarmah et al. (2010). To the best of the author’s knowledge, this study is the first investigation addressing the remediation of biochar for the reduction of manure-borne hormonal pollution in soil and water. The present study’s primary objective was to monitor the fate and transport of the two manure-borne steroidal sex hormones (E2 and E1) in sandy soil over a 45-day period. Poultry manure was applied as fertilizer to outdoor lysimeters irrigated with different levels of simulated rainfall. The study’s secondary objective was to evaluate the remediation potential of slow-pyrolysis biochar topsoil amendments as a novel remediation technique.

5.3 Methodology

The detailed methodology information including the experimental set up, the soil and biochar physical and chemical properties, the characteristics and analysis of poultry manure, extraction of hormones from soil, leachate samples and hormonal content analysis and mass balance calculation are presented in Chapter 4, section 4.3.

5.3.1 Separation, identification and quantification of E2 and E1

The specific concentration of each estrogen hormone standard solution was prepared in 50/50 (V/V) purified Milli-Q water and HPLC-grade acetonitrile. The detection and concentration tracing of these two estrogenic hormones were carried out through a Zorbax Eclipse Plus C18 column (150 × 4.6 mm) with particle size of 5 μm (Agilent, Santa Clara, CA). The best detector response for the identification of both E2 and E1 was
at 200 nm with the retention of 8 min (E2) and 13 min (E1). The mobile phase used in this process was a volumetric mixture of ratio of 60% of purified Milli-Q water and 40% HPLC-grade acetonitrile, with a flow rate of 1 mL/min with the injection volume of 100 μL for isocratic analysis of estrogens. Prior to each analysis, the mobile phase was filtered by 0.45 μm membrane filters and degassed. The column temperature was kept constant at 25 °C during all analysis. To identify the retention time and the peak geometry of each compound, the injection of pure standards of each hormone, with a different range of concentrations (5-0.001 mg L⁻¹), was performed. By using the external standard peak areas from chromatogram, at specific concentrations, a seven-point calibration curve equation for each compound was based on the linear regression in Excel (Microsoft Office Software). The detection response of the instrument to the lowest concentration of each hormone was different. The limit of detection for the progesterone was 0.001 mg L⁻¹ and 0.003 mg L⁻¹ for E2 and E1. 3.80±0.12 (μg/Kg) of E2 and 0.36±0.0.015 (μg/Kg) of E1 was quantified in the analyzed poultry manure samples.

5.4 Statistical Analysis

The hypothesis of significant effect of the remediation ability of biochar to reduce the hormonal pollution in soil and water was statistically analyzed by the spatial and temporal repeated measures model, using PROG GLM, in SAS v.9.2 (SAS Institute Inc, 2010)

5.5 Results

5.5.1 Estrogen distribution in soil and water samples

The spatial-temporal distribution concentration of E2 and E1 in soil under the two treatments, soil and soil-biochar, at four sampling depths including the surface, 15, 35 and 65 cm over 46 days are presented in Figs 5.1 and 5.2. Significant differences (p<0.05) were found in analyzed concentrations of E2 and E1 in soil samples between the soil and soil-biochar treatments with respect to both time and depth. In both treatments, the hormone concentrations followed a decreasing trend over time in the soil surface. In the lysimeters receiving biochar, the highest concentrations of estrogens were found in the soil surface after the first irrigation at day 0. The measured E2 concentration at the
soil surface, in the presence of biochar, was $308.10\pm6.85\ \mu g/kg$. However, in the lysimeters under the soil treatment, the average E2 concentration in the soil surface at day 0 was measured $64.14\pm6.85\ \mu g/kg$ which was almost five times lower than the biochar treatment, indicating the rapid leaching and movement of E2 in this treatment, as the highest concentrations of hormone was found at depths 35 cm and 15 cm at day 0 with measured concentrations of $52.28\pm11.48\ \mu g/kg$ and $40.69\pm6.88\ \mu g/kg$ respectively. The concentration of E1 in the soil surface, amended with biochar, at day 0, was measured $36\pm3.89\ \mu g/kg$ whereas the highest concentration of E1 in the soil-only treatment, found in the surface, was $21.93\pm4.75\ \mu g/kg$. The 40% lower concentration of E1 in the soil treatment can be considered as potential rapid downward movement of E1 in the sandy soil or a good indication of fast degradation of estrogens in natural soil. The concentration of E1 at depths of 15 cm and 35 cm under the soil treatment was measured $7.34\pm1.96\ \mu g/kg$ and $3.1\pm0.94\ \mu g/kg$ whereas $3.7\pm0.71\ \mu g/kg$ and $1.27\pm0.22\ \mu g/kg$ of E1 was found at depths 15 cm and 35 cm respectively, under the biochar treatment at day 0.

The analyzed concentrations of estrogens measured at depths of 15 cm and 35 cm in lysimeters under the biochar treatment, were found to be significantly (P<0.05) lower than the hormone concentrations measured in the lysimeters under soil treatment, even after the first irrigation. This demonstrates the effective sorption ability of slow pyrolysis biochar for the retention of hormones in soil. The concentration distribution of E2 and E1 through depth and time under biochar treatment was significantly different from soil treatment (P<0.05). In lysimeters without the 1% biochar amendment, the soil surface concentration of E2, introduced by the application of manure, was rapidly decreased more than 50% in 3 days, and after 7 days, only 20% of the initial concentration was found in the soil surface. Before the second irrigation on day 15, the concentration of E2 was measured to be $3.38\pm0.41\ \mu g/kg$ in the samples collected under soil treatment, indicating that only 5% of the primary hormone’s concentration was available on the soil surface. Due to the role of the texture and physical properties of the soil as two other governing factors affecting the fate and transport of hormones in soil, the higher sand content in the upper soil profile would lead to less sorption and more E2 would be dissolved in the aqueous phase as a result, causing more leaching of the hormones to lower depths. However, the longer persistence of hormones in the lower part of the soil.
profile could be due to less oxygen availability and less biological activities (Fan et al., 2007). However in the presence of biochar, after 7 days, almost 45% of the initial E2 concentration was still available and more than 20% of the initial hormone’s concentration was measured on day 15 before the second irrigation. On day 46, one day after the application of the last irrigation, more than 2% of the initial concentration was detected in the soil surface of lysimeters under soil treatment. Based on the analyzed concentrations of E2 in soil samples at lower depths, the major spatial distribution of E2 in the initial 15 days was observed at depth 35 cm in the range of 42.48-52.28 μg/kg and at a depth of 15 cm in the range of 15-40.69 μg/kg in lysimeters under soil treatment with an ascending trend followed by a gradually descending pattern at both depths. The same trend was observed in the lysimeters receiving biochar; however, the lower depth’s concentrations of hormones were almost half of the detected ones in soil treatments, demonstrating the significant difference between biochar treatment and soil treatment.

After the second irrigation on day 15, a detectable concentration of E2 was found at depth 65 cm under both treatments, indicating the potential of estrogenic hormones to leach downward in the soil column. However, the E2 concentration found at depth 65 cm with the biochar treatment was almost 5 times lower than the lysimeters under soil treatment indicating a significant ability of the biochar to hold the hormones near the soil surface. Significant differences (p<0.05) in analyzed concentrations of E2 in soil with respect to both time and depth were found through statistical analysis. A highly significant interaction between time and depth was found, since the initially increasing trend following a descending trend in the spatial distribution of concentrations at lower depths was observed over the time period. After the application of manure, the initial surface concentration of E1 under the soil treatment decreased almost 50% after one day and only 18% of the preliminary E1 concentration was detected in the surface at day 7. The analyzed concentration of E1 after 15 days was 2.14±0.85 μg/kg demonstrating only 10% bioavailability of E1 hormones in the soil surface.

In contrast to the E2 distribution in the soil surface under biochar treatment, the E1 concentration decreased to less that 50% of the initial concentration after one day as compared to the hormone concentration measured under soil treatment; however, the E1
content of the surface soil was still significantly higher in the presence of biochar when compared to soil alone. The hormone content at the soil surface did not significantly change from day 3 to day 16, after the second irrigation, indicating the biochar’s ability to hold the residual hormones in the soil surface. 23 days after the application of poultry manure and two irrigations, only 7% of the initial E1 was detectable in the soil samples for the depth 0 cm; however, more than 18% of the initial E1 content was still available in the soil surface containing, 1% slow pyrolysis biochar. After applying the third irrigation on day 30, the detected concentration of hormones was measured 0.72 ± 0.27 (μg/kg) representing only 3% E1 available in the soil, whereas the analyzed concentration of soil samples from biochar treatment, 3.180±0.39 μg/kg indicated almost 8.5% availability of E1 in the soil surface. After the last irrigation on day 45 and even one day later, we were not able to detect any E1 in the soil samples.

Monitoring the analyzed concentrations of E1 in soil samples at lower depths showed a major spatial distribution of E1 at depth 15 cm in the initial 15 days, ranging from 4.92 to 9.071 μg/kg and at a depth of 35 cm in the range of 1.072-5.79 μg/kg in lysimeters under soil treatment with an upward trend followed by a gradually decreasing trend at both depths. The same trend was observed in the lysimeters receiving biochar; however, the E1 concentration was significantly lower than the soil treatment at lower depths of the soil profile, ranging from 1.077-3.71 μg/kg at a depth of 15 cm and 0.25-1.27 μg/kg at a depth 35 cm, demonstrating the significant difference between biochar treatment and soil treatment. Detectable concentrations of E1 were found at depth 65 cm under soil treatment after the second irrigation on day 16 with the highest concentration of 1.09 μg/kg; however, E1 was only detectable in samples collected at depth 65 cm under biochar treatment on day 16 with a concentration of 0.0414±0.0211 μg/kg one day after the second irrigation, and day 23 with a concentration of 0.19±0.0591 μg/kg. The E1 concentration was found under the detection limit after the last irrigation on day 45.

The association of hormones with dissolved or colloidal fractions of soil and/or manure can be considered as one possible explanation for hormone persistence and greater than expected mobility in soil (Cox et al., 1997). The sorption behavior of estrogens could be due to the reactive phenolic group in these hormones which can interact with humic acids
or mineral surfaces via hydrogen and covalent bonds (Yu et al., 2010). Approximately 50-73% of E2 and its metabolites can be bonded to humic substances, which represents more than 50% of the organic matter of soil (Finlay-Moore et al., 2000). Humic substances can increase the mobility of sorbed contaminants by acting as mobile, or flowing, solid phase. However, humic substances have a sorption ability similar to the stagnant solid phase; they have the mobility potential similar or greater than the mobile aqueous phase (McGechan and Lewis, 2002). Non-hydrophobic sorption interactions of E2 are hypothesized by Yu et al. (2004) by the interaction of the phenolic group of estrogens with humic acids or mineral surfaces via hydrogen and covalent bonding.

5.5.2 Temporal stratification of E2 and E1 in leachate samples

After each irrigation, the leachate samples were collected on day 0, 15, 30 and 45; the estrogen content of each sample was quantified using HPLC analysis. The average concentration of E2 and E1 in the leachate samples under the two treatments over time is demonstrated in Fig 5.3. However, a significant trace concentration (μg/L) of both hormones was found in the leachate samples; the detected concentration of hormones in water samples was relatively lower than hormones concentrations measured in soil samples. This may be due to the high sorption affinity of estrogenic hormones to organic matters available in the upper soil profile in the lysimeters. The sorption of hormones to clay or dissolved organic matter, known as mobile soil particles, can enhance steroid transport via runoff or leaching (Das et al., 2004; Yamamoto et al., 2003).

Statistical analysis demonstrated a significant difference between the hormone’s concentrations measured in the leachate samples under the two treatments. The initial concentration of 37.99±0.26 μg/L of E2 and 15.16±0.1 μg/L of E1 were found in leachate samples under the soil treatment after the first irrigation at day 0, whereas only 5.79±0.04 μg/L of E2 and 5.71±0.1 μg/L of E1 were measured in the leachate collected under biochar treatment. In both treatments, the hormone’s concentrations measured in leachate samples were increased over time; for example, the highest concentration of estrogens were found after the last irrigation on day 45 where the concentrations of E2 were 143.68±1.05 μg/L and 40.53±0.28 μg/L in the soil and biochar treatments, respectively. The concentration of E1 was 101.58±0.72 μg/L under soil treatment and 25.85±0.18 μg/L.
under biochar treatment. The manure application rate, precipitation events demonstrated a significant correlation to the detected concentrations of hormones in the leachates of lysimeters (Thompson et al., 2009)

5.5.3 Mass balance

The mass balance analysis was performed for both estrogens using the measured concentrations of E2 and E1 in the soil and water samples for all eleven sampling days. Table 5.1 and 5.2 represent the residual hormone content in different depths of the soil profile and collected leachate samples through time for the two treatments. The total mass of both estrogens dissipated in the soil profile rapidly even after one day. More than 35% of the initial E2 mass of (1520 ±1.256 μg) was decreased, which was introduced to the soil surface one day before the first irrigation on day 0. The initial E2 mass in the lysimeters under soil treatment was 984.3±0.78 μg, which was reduced by as much as 54%, one day after the first irrigation, and two days after the application of manure. The initial E2 mass content of the soil profile under the biochar treatment was calculated to be about 1496.3±0.88 μg. In spite of the large E2 mass loss under soil treatment, even before the first irrigation, only 1.5% of the hormones were lost in the lysimeter’s soil profile after the first irrigation in the lysimeters receiving biochar as a soil amendment. A major mass of E2 hormones were persistent in the surface of the soil in the presence of biochar; however, a large portion of the E2 mass was found between 15 and 45 cm in the soil profile in the lysimeters under soil treatment. The comparison of mass balance data for each depth of the soil profile demonstrates the significant retention ability of biochar for sorption of hormones in the soil surface and decreasing the leaching rate of hormones toward subsurface water. For instance, only 5.43 μg of E2 was detected in the surface under the soil treatment whereas the E2 mass under biochar treatment measured 157.09 μg in the topsoil after the second irrigation on day 15. The E2 level found in the leachate samples collected under the soil treatment were almost 7 fold greater than the biochar treatment after the first irrigation. The E2 content of the water samples, receiving biochar, was 3 times lower than E2 recovered in the water samples collected under soil treatment after the last irrigation on day 45.
One day after the incorporation of manure and the first irrigation only 61.78±0.51 μg of E1 was found in the topsoil of lysimeters under soil treatment indicating more than 58% reduction of initial E1 (147.17±0.854 μg) whereas more than 80% of E1 was still remaining in the soil surface in the presence of biochar. Similar to the mass distribution of E2, major mass of E1 was persistent in the surface of soil in the presence of biochar; however, large portion of E2 mass was found in between 15 and 45 cm in the soil profile in the lysimeters under soil treatment. 3.44±0.84 μg of E1 was measured in the surface after 15 days and more than 69 μg was found at the depth of 65 cm after the application of second irrigation under the soil treatment whereas 11.57±0.34 μg of E1 was remaining in the topsoil in the presence of biochar. 3-fold greater mass of E1 was quantified in the leachate samples collected under soil treatment, as compared to biochar. After the last irrigation on day 45, the E1 content of water samples receiving biochar was found 4 times lower than E1 recovered in the water samples collected under soil treatment. These results indicate a better perspective for remediation by biochar as a soil amendment where biochar potentially provides a heterogeneous sorption site consisting of pre-existing meso- and micropore cavities with different pore sizes and pore surfaces, which results in the heterogeneity of nonspecific sorption-site energies where van der Waals forces play the dominant role as the solute-sorbent interactions (Xing et al., 1996).

### 5.5.4 Degradation kinetics of Estrogens under the field condition

The degradation kinetics of both E2 and its metabolite, E1, in the two treatments were determined based on the first-order decay (Johnson, 2001) with the governing model as follows:

\[
\frac{dC_t}{dt} = -kt \quad (3)
\]

Integrating Eq. (3) gives

\[
C_t = C_o e^{-kt} \quad (4)
\]

Where \(C_t\) is the residual hormone concentration at time \(t\), \(C_o\) is the initial concentration, and \(k\) is the degradation rate constant.
Fig 5.1 Concentration of E2 in the soil and soil-biochar treatments at four sampling depth of lysimeter receiving poultry manure (log scale used for Y axis plotting)
Fig 5.2 Concentration of E1 in the soil and soil-biochar treatments at four sampling depth of lysimeter receiving poultry manure (log scale used for Y axis plotting)
The natural logarithm of mass/initial mass of each hormone versus time was plotted where the slope of the linear graph represented the degradation rate constant (k), which was used to calculate the half-lives of estrogens in soil. The values of model-fitting parameters are presented in Table 5.3, which demonstrates that the degradation of estrogenic hormones in soil under field conditions can be explained by the first-order rate degradation model. The degradation rate constant of E2 under biochar and soil treatment was 0.0034 h\(^{-1}\) and 0.0034 h\(^{-1}\) respectively.
A wide range of first-order degradation/transformation rate constants of E2 (0.0006 h⁻¹) (Fan et al., 2007) and 0.252 h⁻¹ (Layton et al., 2000) have been reported. The half-life of E2 was determined as 8 days under both treatments while the half-life of E1 was calculated 9 days under the biochar treatment and 7 days under the soil treatment indicating that estrogenic hormones are moderately persistent in the natural matrix under field conditions. However, several studies reported a short half-life for estrogens in soil and water media; for example, Colucci et al. (2001) and Jacobsen et al. (2005) reported the degradation of E2 within 24 h which was accompanied by an accumulation of E1, which subsequently degraded within 72 h. Based on the fitting model data, a similar degradation pattern was observed for both hormones. However, the moisture content, redox conditions and temperature demonstrated an influential effect on the degradation of both hormones (Xuan et al., 2008). They reported the degradation dependency on soil temperature where they found at 15 °C, the rate constant (k’) and the half-life were 0.141±0.008 day⁻¹ and 4.9 days, respectively. When the temperature was increased to 25 °C, the rate constant increased to 0.750 day⁻¹, which is 4.3 times higher than at 15 °C and correspondingly, the half-life was reduced to 0.92 days.

Steroid hormones are considered as a source of energy for bacteria in a redox (reduction/oxidation) reaction. These steroids become a carbon source for cell growth by metabolization (Donova, 2007; Korom, 1992). Various microbial species including Mycobacterium, Arthrobacter, Bacillus, PSedumonas, Escherichia and Micrococcus have been introduced as the main transformation processors of steroid hormones in environmental media (Fernandes et al., 2003; Jenkins et al., 2004; Malaviya and Gomes, 2008). Danielsson and Zhang (1996) indicated that the biotransformation of estrogens takes place until log Kow reaches 3.0-3.5 but at higher log Kow values, sorption and solid phase partitioning would be the dominant procedure governing the behavior of estrogens. The redox conditions (aerobic vs. anaerobic) can significantly influence the degradation of hormones whereas the incorporation of manure containing sex hormones in the topsoil would result in soil conditions potentially remaining anoxic for a longer period of time and therefore, would retard the degradation of hormones with a greater chance of bioavailability (Ying and Kookana, 2009). However in this study, the effect of redox conditions was not evaluated. Coexistence of veterinary antibiotics with estrogenic
hormones in animal waste and animal manure-fertilized agricultural soil may greatly reduce the biodegradation of estrogens in the environment by affecting microorganisms at very low the toxic dose. Therefore the influence of the coexistence of other manure-borne organic contaminants should be considered in the evaluations of the fate and transport of estrogenic hormones in the environment.

5.5.5 Role of biochar

Significantly lower concentrations of E2 and E1 in soils receiving the biochar treatment, has confirmed the hypothesis of this study, which proposed the effective retention capability of biochar as a soil-amendment for reducing manure-borne hormonal pollution in soil and water. Recent studies, investigating the sorption ability of different types of biochars for the reduction of various emerging contaminants, including antibiotics, herbicides, pesticides and heavy metals, have reported a similar positive retention capability of biochar (Chun et al., 2004; Lehmann et al., 2003; Sarmah et al., 2010; Spokas et al., 2009; Uchimiya et al., 2011; Yu et al., 2011; Zheng et al., 2010). Higher sorption affinity and stronger desorption resistance of biochar is considered as a function, which can regulate the aqueous concentration of these contaminants, and as a result, affect leaching, the environmental bioavailability and degradation of these hormones. The chemical and structural properties of the sorbent (e.g. the particle size distribution, specific surface area and total porosity) and the physic-chemical properties of the sorbate (e.g. hydrophobicity or charge characteristics)(Kookana, 2010) are the dominant functions governing the sorption of organic contaminants to the principal sorbent in the soil (e.g. Soil organic matter and clay particles) or other organic sorbents such as black carbon or biochar. The feedstock, pyrolysis conditions and temperature, carbon content and degree of aromatic condensation (Sarmah et al., 2010) are considered as major variables influencing the sorption affinity and retention capacity of different types of biochars and their resistance to the desorption of organic contaminants in the soil-biochar media.
5.6 Conclusion

A lysimeter study was carried out in order to evaluate the retention ability of slow pyrolysis biochar as soil-amendment to reduce the manure-borne hormonal pollution in soil and water under field condition. The fate and transport of two female sex hormones, including E2 and E1 were investigated in the soil receiving poultry manure under two different treatments, with and without biochar, over 45-days period. The significant difference between the spatial-temporal distribution concentration of E2 and E1 in soil under the two treatments, soil and soil-biochar, at four sampling depths, including the surface, 15, 35 and 65 cm over, 46 days has confirmed the hypothesis of this study proposing the highly effective retention capability of biochar as a soil-amendment for reducing the manure-borne hormonal pollution in soil and water. Higher sorption affinity and stronger desorption resistance of biochar is considered as functions which can regulate the aqueous concentration of these contaminants, and as a result, affect leaching, the environmental bioavailability and degradation of these hormones. Therefore, it can be proposed that the potential of biochar is important not only as an organic soil amendment for improving the soil quality and crop production but also as a novel remediation technique for better sustainable conservation of water resources. The sorption behavior of organic contaminants is influenced by the aromatic carbon backbone in the porous, amorphous structure of biochar. The investigation regarding the remediation ability of biochar is limited and more detailed studies, specifically temporal field-scale, are required. The results of the present lysimeter study have highlighted the significant retention ability of biochar for reducing the manure-borne hormonal pollution in agricultural soil media. From an environmental and agricultural perspective, biochar can be proposed as a novel, clean and feasible remediation technique. However, detailed, long term studies are required to investigate the competitive sorption ability of biochar in the simultaneous presence of different chemicals in the soil media.
Table 5. Mass balance profile of E2 (μg) in the lysimeter soil profile and leachate samples over the 45 day period

<table>
<thead>
<tr>
<th>Soil profile (cm)</th>
<th>Soil treatment</th>
<th>0-5</th>
<th>5-15</th>
<th>15-45</th>
<th>45-70</th>
<th>Cum. leachate</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
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<td>180.69</td>
<td>170.93</td>
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<td>**</td>
<td>* 0.27</td>
<td>948.31±0.78^a</td>
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<tr>
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<td>**</td>
<td>* 0.18</td>
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<td>0.62</td>
<td>0.27</td>
<td>**</td>
<td>**</td>
<td>-</td>
<td>0.89±0.55</td>
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<th>Soil profile (cm)</th>
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</tr>
</tbody>
</table>

*Mass of E2 (μg) in total collected volume of leachate samples after each irrigation

**Below the detection limit of E2 in analytical process

^a Standard deviation
Table 5.2 Mass balance profile of E1 (μg) in the lysimeter soil profile and leachate samples over the 45 day period

<table>
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<th>Soil profile (cm)</th>
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<td>61.78</td>
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<td>36.33</td>
<td>**</td>
<td>-</td>
<td>129.09± 0.58a</td>
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<tr>
<td>1</td>
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<td>30.19</td>
<td>30.31</td>
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<td>**</td>
<td>72.92± 0.44</td>
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</tr>
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<td>1.54</td>
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<td>39.64± 0.74</td>
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<td>129.09± 0.58a</td>
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<td>0.71</td>
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<th>5-15</th>
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<td>Day</td>
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<td></td>
</tr>
</tbody>
</table>

*Mass of E1 (μg) in total collected volume of leachate samples after each irrigation

**Below the detection limit of E1 in analytical process

*a Standard deviation
Table 5.3 Linear regression and half-lives of E2 and E1 under the two treatments

<table>
<thead>
<tr>
<th>Biochar treatment</th>
<th>Regression slope (K)</th>
<th>Coefficient of determination ($r^2$)</th>
<th>Half-life (days)</th>
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<td>8</td>
</tr>
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<td>Estrone</td>
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<td>0.85</td>
<td>9</td>
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</tbody>
</table>

<table>
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<th>Regression slope (K)</th>
<th>Coefficient of determination ($r^2$)</th>
<th>Half-life (days)</th>
</tr>
</thead>
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<td>8</td>
</tr>
<tr>
<td>Estrone</td>
<td>0.0919</td>
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<td>7</td>
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</tbody>
</table>

Fig 5.4 Natural logarithm of ass/initial mass ($m/m_0$) of E2 and E1 versus time under two treatments
PREFACE TO CHAPTER 6

In the previous chapter, the significance of biochar was observed for reducing the manure-borne estrogenic contamination in soil and water samples in lysimeters. Estrogens are the dominant naturally manure-borne female sex hormones.

In this chapter, the environmental fate and transport of the manure-borne estrogens was investigated under two different treatments, with and without biochar, over a 45-day period using a field-scale lysimeter study where liquid swine manure was applied to the topsoil as an organic fertilizer.

To date, this is the first field study highlighting the influence of the retention potential of slow-pyrolysis biochar on the environmental behavior, fate and transport of manure-borne estrogens in soil media. This research will help us understand the role of biochar in improving the water quality and can be potentially used in agricultural fields to mitigate the concerns of manure-borne hormonal pollution.

Research papers based on the chapter:

S.Alizadeh, F.Gobbi, S.O.Prasher. Role of Slow-Pyrolysis Biochar Amendment on the Fate and Transport of Estrogenic Hormones in Liquid Swine Manure
Chapter 6
Role of Slow-Pyrolysis Biochar Amendment on the Fate and Transport of Estrogenic Hormones in Liquid Swine Manure

6.1 Abstract

The primary objective of this study was to evaluate the remediation potential of slow-pyrolysis biochar as topsoil amendments to reduce pollution from two manure-borne steroidal sex hormones, 17β-estradiol (E2) and estrone (E1), in a sandy soil. Liquid swine manure was applied as a fertilizer to outdoor lysimeters irrigated with different levels of simulated rainfall. To the best of the author’s knowledge, this study is the first investigation addressing the use of biochar for the reduction of manure-borne hormonal pollution in soil and water. A significant difference was observed between the spatial-temporal distribution concentration of E2 and E1 in soil under the two treatments, soil and soil-biochar, at four sampling depths including the surface, 15, 35 and 65 cm over 46 days. The results of this study demonstrated that the higher sorption affinity and stronger desorption resistance of biochar should be considered as factors regulating the aqueous concentration of these contaminants and as a result, affect leaching, the environmental bioavailability and degradation of these hormones.

Keyword. Swine manure, 17β-estradiol, estrone, biochar, lysimeter

6.2 Introduction

The occurrence, environmental exposure and the fate and transport of a new class of toxic and high-risk organic contaminants, with endocrine disrupting properties, have become the foremost concern in environmental studies. Classified as endocrine disrupting compounds (EDC), these toxic chemicals are able to alter or interfere with the metabolism and biosynthesis of endogenous hormones and modulate the functions of the endocrine system (Colucci et al., 2001; Sonnenschein and Soto, 1998). This can lead to chronic toxicity and adverse, long-term health issues (e.g. carcinogenicity, mutagenicity or teratogenicity) at concentrations as low as ng/L (Choi et al., 2004). Although various categories of synthetic and industrial compounds including pesticides, polychlorinated
biphenyls (PCBs) and nonylphenols (Gould et al., 1998; Kavlock et al., 1996) have been classified as major EDCs. The natural steroidal sex hormones (i.e. 17β-estradiol (E2), estrone (E1), testosterone, progesterone) are also cause of concern due to their detrimental and carcinogenetic effects.

Several cases of aquatic intersexuality, wildlife biological disruption and development reproductive abnormalities caused by steroid hormones have been considered as evidence of environmental occurrence and the presence of steroidal sex hormones. The feminization of male fish or the masculinization of female fishes (Orlando et al., 2004; Soto et al., 2004; Vajda et al., 2011; Vajda et al., 2008) or the reproductive biology alteration of wild fathead minnows (Pimephales promelas) and rainbow trout at aquatic E2 concentrations as low as 1-10 ng/L E2 and 25-50 ng/L of E1 (Fang et al., 2003; Young and Borch, 2009) indicates that there is hormonal environmental contamination. Expanding anthropogenic activities, wastewater plant (WTPs) discharges and agricultural land management practices, specifically the application of animal manure and other biosolids (e.g. sewage sludge) on agricultural land have been identified as dominant gateways for environmental exposure to steroid hormones. The urine and feces of all species, sexes and classes of farm animals are major sources of manure-borne steroid hormones (Lai et al., 2002). Liquid swine manure, the most widely applied organic fertilizer in the North America, is injected into the top 10 cm of agricultural soil (Zitnick et al., 2011), and this has been identified as a principal source of bioactive levels of natural steroidal sex hormones, including E2, E1, testosterone and progesterone.

The total estimated mass of steroids in the environment in China is reported as 139,000 kg/year from swine farms (Liu et al., 2012). Combalbert et al. (2012) demonstrated the release of significant concentrations of E1 and E2 into the environment through the traditional spreading of stored pig manure. In the survey of hormone activities in municipal biosolids and animal manure, Lorenzen et al. (2004) reported the highest detected levels of steroid hormones (5965 ng/g dry weight) in manure from finishing pigs and the lowest level in manure from steers (0.43 ng/g dry weight). In a similar study, the excretion of steroid hormones from swine and cattle manure in European Union and the
United States was reported by Lange et al. (2002a). The sex, age, and reproductive status and species of animals was found to effect the levels of released hormones by animals; another influential factor on these levels was influenced by the diet and veterinary treatments of the animals (Lorenzen et al., 2004). Different animals excrete the steroid hormones in various ways. More than 58% of estrogens excreted by cattle are found in their feces whereas swine and poultry excrete estrogens mostly in urine (96% and 69%, respectively) (Hanselman et al., 2003).

Although livestock manure, specially poultry manure, is considered as a major source of estrogenic hormones contributing to surface waters (Peterson et al., 2000), the greatest potential environmental production and contribution of natural estrogenic compounds has been reported by manure from swine (Sus scrofa domesticus) gestation and farrowing facilities (Zitnick et al., 2011). Swine slurry is a complex mixture of suspended and dissolved organic and inorganic substances, including nutrients and contaminants (Aust et al., 2009; Leenheer and Rostad, 2004). More than 50% of the organic matter in liquid manure is present as Dissolved Organic Carbon (DOC) and Colloidal Organic Carbon (COC) which is potentially the most mobile fraction where the association of estrogenic hormones with DOC/COC fractions in soil and sediments indicates the significant role of the fraction on their fate and transport in soil matrix (Aust et al., 2009). It has been reported that more than 95% of estrogens in liquid swine manure are deconjugated and dissolved, which is greater than that of other animal manure sources (Hutchins et al., 2007).

Known as weakly acidic and hydrophobic compounds with high sorption affinity to soil organic matters, several laboratory studies have demonstrated the rapid dissipation of steroid hormones with short half-life under aerobic conditions (Casey et al., 2005; Das et al., 2004; Lee et al., 2003). However, several recent investigations (Arnon et al., 2008; Kjær et al., 2007; Kolodziej et al., 2004) have demonstrated the significant manure-borne steroid hormonal contamination of the surface and groundwater resources from the runoff effluent from the agricultural fields receiving manure. Herman and Mills (2003) reported frequent detection of estrogens in the zone beneath a manure-fertilized corn (Zea mays)
field in lysimeter leachate. It is estimated that at least 90% of the total estrogen contributed to the environment occurs as a result of the application livestock manure (Maier et al., 2009). The environmental availability of excreted steroidal estrogens in the urine and feces of livestock, irrespective of their sex and age (Khanal et al., 2006), have been identified as the predominant source of adverse long term ecological and biological impact.

6.2.1 Biochar, a novel environmental remediation technique

Due to their highly adsorptive properties, the in-situ application of organic carbonaceous materials as a soil amendment has been proposed as a multi-purpose and financially-feasible approach to reduce the bioavailability and bioaccumulation of organic contaminants. The strong sorption affinity of carbon-rich amendments, such as black carbon, activated carbons and biochar for poly-aromatic hydrocarbons and other categories of organic contaminants, may make it possible to use them as a novel bio-remediation technique. The thermo-chemical conversion of biomass and biological residues (e.g. wood, poultry litter, crop residues, etc.) in the absence of oxygen (or partially combusted in the presence of a limited oxygen supply) is known as pyrolysis and leads to carbonizing organic material into biomass and results in the production of energy-rich and valuable end-products (Meng et al., 2013), including bio-oil, combustible gases and a fine-grained carbonaceous residue, named biochar.

Different pyrolysis conditions including the heating rate and the speed of the pyrolysis leads to the production of biochars with specific structural and physio-chemical properties. Slow pyrolysis biochar is the product of the decomposition of biomass at relatively low temperatures (300-500 °C) with a long heating time and a heating rate less than 10 °K/min whereas fast pyrolysis takes place over a short time at high temperatures (700-900 °C) with a heating rate of more than 1000 °K/min (Mohan et al., 2006). Biochar with amorphous structure, containing nano-scale condensed aromatic rings with a crystalline structure and therefore the strong sorption affinity, high specific surface area and resistance to bio-decomposition of biocar offers an approach to engineer the natural process in order to fulfill an environmental remediation of inorganic contaminants (e.g.
heavy metals) and hydrophobic organic contaminants (Beesley et al., 2011). The retention capacity of biochar on the mobility, bioavailability and toxicity of heavy metals and PAHs is reported by Beesley et al. (2011). The reduced pore-water concentration of heavy metals and the resulting effectiveness of biochar on the mobility of copper and lead and the plant uptake of heavy metals was assessed by Karami et al. (2011). Several studies (Rondon et al., 2005; Spokas et al., 2009; Troy et al., 2013) demonstrated the significant role of biochar as a soil amendment or reported that its use resulted in the reduction of greenhouse gas emissions. Yu et al. (2011) investigated the sorption capacity of biochar on different types of pesticides, including triazine and acetamiprid. The only investigation to evaluate the sorption capacity of biochar for estrogens was conducted by Sarmah et al. (2010).

The application of biochar lowers the bulk density of the soil which potentially improve the soil aeration and therefore would accelerate the decomposition of organic contaminants by microorganisms or their enzymes (Brewer, 2012). Simultaneously the strong adsorbing ability of biochar might also make the sorbed contaminants less susceptible to enzymatic attack. Several studies (Enders et al., 2012; Novak et al., 2009; Oguntunde et al., 2004; Van Zwieten et al., 2010; Warnock et al., 2007) reported the improved soil physio-chemical properties as a result of biochar amendment. The presence of biochar in topsoil increases the nutrient availability and results in better fertility. Increased pH levels, higher water holding capacity, and water infiltration are some examples of the enhanced soil physical properties by applying biochar. Several laboratory scale studies investigated the remediation of organic contaminant bioavailability by the application of activated carbon and biochar (Cho et al., 2009; Herman and Mills, 2003; Millward et al., 2005; Sun and Ghosh, 2008; Zimmerman et al., 2004); however, there is a paucity of information regarding the in-situ field-scale retention ability of biochar. The only investigation to evaluate the sorption capacity of biochar for estrogens at the laboratory scale was conducted by Sarmah et al. (2010). The primary objective of this study was to evaluate the potential of slow-pyrolysis biochar topsoil amendments in reducing the transport of the two manure-borne steroidal sex hormones, E2 and E1, in sandy soil over a 45-day period. Liquid swine manure was applied as a fertilizer to
outdoor lysimeters irrigated with different levels of simulated rainfall. To the best of the author’s knowledge, this study is the first investigation addressing the remediation of biochar for the reduction of manure-borne hormonal pollution in soil.

6.3 Methodology

The detailed methodological information, including the experimental setup, the soil and biochar physical and chemical properties, the characteristics and analysis of swine manure, extraction of hormones from soil, leachate samples and hormonal content analysis and mass balance calculation were presented in Chapter 4, section 4.3. The details on soil and leachate extracts and analysis are reported in Chapter 5, section 5.3.1. 0.673±0.075 (μg/Kg) of E2 and 1.40±0.15 (μg/Kg) of E1 was quantified in the analyzed swine manure samples.

6.4 Statistical Analysis

The hypothesis of significant effect of the remediation ability of biochar to reduce the hormonal pollution in soil and water was statistically analyzed by the Special and temporal repeated measures model using PROG GLM in SAS v.9.2 (SAS Institute Inc, 2010)

6.5 Results

The environmental bioavailability and degradation of steroid sex hormones can be affected by the high sorption affinity and strong desorption resistance of biochar. The hypothesis of this investigation proposed the effective remediation potential of biochar as a soil-amendment for reducing manure-borne hormonal pollution in soil and water, which was confirmed by the significant difference (p<0.05) found between quantified spatial and temporal estrogen mass stratification under biochar and soil treatments.

6.5.1 Estrogen distribution in soil and water sample

The spatial-temporal distribution concentration of E2 and E1 in soil under the two treatments, at four sampling depths including the surface, 15, 35 and 65 cm over 46 days are presented in Figs 6.1 and 6.2.
6.5.2 Spatial and temporal stratification of E2 and E1 in soil samples

The quantified concentrations of E2 and E1 in soil samples were found to be significantly different (p<0.05) under soil and biochar treatments with respect to both time and depth. Based on the spatial distribution of E2 and E1 in the soil profile, a descending trend of the concentration for each was monitored in the topsoil over time. The highest concentration of each hormone was measured in the soil surface of the lysimeters receiving 1% slow pyrolysis biochar, after the first irrigation at day 0, whereas the remaining concentrations of hormones were distributed at lower depths. One day after the injection of liquid swine manure to the 10 cm topsoil and the first irrigation on day 0, 45.26±12.97 μg/kg of E2 and 243.90±3.55 μg/kg of E1 were quantified in the topsoil samples, collected from the lysimeters under soil treatment; however, the average concentrations of E2 and E1 under biochar treatment were found to be 103.02±10.37 μg/kg and 2425.29±16.47 μg/kg, respectively. The significant difference (p<0.05) found between the average concentration of estrogens under each treatment one day after the application of manure indicates the rapid movement of the hormones in the sandy soil and downward leaching in the soil column.

The concentration distribution of hormones at the lower depths of the soil column under the soil treatments demonstrated the highest concentrations of hormones at depth 35 and 15 cm at day 0 where 2.76±0.99 μg/kg of E2 and 4.41±0.77 μg/kg of E1 was quantified at depth 15 cm and their concentrations were 4.58±0.65 μg/kg and 4.958±0.35 μg/kg respectively at depth 35 cm. However, the concentration of E2 at 15 and 35 cm of the soil profile of the lysimeters receiving 1% slow pyrolysis biochar were approximately 50% lower as compared to a similar soil profile in lysimeters under soil treatment. Only 25% of the initial measured E1 (0.929±0.11 μg/kg) was found at depth 15 cm under biochar treatment and at 35 cm depth, the E1 concentration was below the detection limit. The significant difference between the analyzed concentrations under the two treatments at the lower depth of the soil profile can be considered as evidence for the effective sorption ability of the slow pyrolysis biochar for the retention of hormones in soil.

The temporal stratification of both estrogenic hormones under biochar treatment was significantly different (p<0.05) from the soil treatment. Almost 50% of the initial surface
concentration of both estrogens was reduced under the soil treatment only three days after the first irrigation and the application of manure. After 7 days, only 17% of the initial concentration E1 and 33% of E2 was found in the topsoil. On the day 15, before the second irrigation, only 13.40±1.29 μg/kg of E2, representing only 28% available E2 and only 3.5% of E1 (8.337± 0.59 μg/kg) were found in the topsoil under the soil treatment. At day 31, one day after the simulation of the third rainfall, only 0.16% of E1 and 12% of E2 was quantified in the surface of the lysimeters. The concentration of both hormones on days 45 and 46 was found to be below the detection limit.

However, under the biochar treatment, different temporal concentration stratification was observed. When compared to the soil treatment data, almost 64% of E1 concentration and 60% of E2 was still available at day 3. More than 24% of initial E1 and 41% of E2 was measured on day 7. Before the application of the second irrigation, on day 15, 201.2±13.17 (μg/kg) of E1 (8% of initial concentration) and 41.49 ± 4.83 (μg/kg) of E2 representing 39% of the initial concentration was observed. On day 31, 21% of E2 and 5% of E1 were still detectable, even though for the next two sampling dates, the quantified concentrations were below the detection limit. As shown in figures, the major spatial distribution of E2 concentration in soil samples at lower depths was observed in the first 15 days of the investigation and at depth 35 cm in the range of 0.823-4.584 μg/kg and at a depth of 15 cm in the range of 1.76-4.031 μg/kg in the lysimeters under soil treatment. An ascending trend of E2 concentration was observed in the first three days, followed by a descending pattern through the next sampling dates where a decreasing concentration trend was observed at depth 35 cm from 4.584±0.653 μg/kg to 0.575±0.078 μg/kg in 23 days. However, after the third irrigation, the E2 concentration increased to 1.812±0.861 μg/kg on day 30 but by the end of the investigation on day 46, no detectable concentration of E2 was quantified.

The concentration of hormones at the lower depths under biochar treatment was almost half of that detected in soil treatments, demonstrating the significant difference between biochar treatment and soil treatment. In the lysimeters receiving 1% biochar, the highest concentration of E2 at depth 15 cm was measured 1.214±0.69 μg/kg at the day 0 which decreased to 0.494±0.079 μg/kg at day 3 and for the other days the concentration of E2
was below the detection limit. After the second irrigation on day 15, a detectable concentration of E2 (2.566±0.324 μg/kg) was found at depth 65 cm on day 16 under soil treatment which decreased to 0.734±0.128 (μg/kg), indicating the potential of estrogenic hormones to leach downward in the soil column. However, the E2 concentration at depth 65 cm with the biochar treatment was not detected. The statistical analysis demonstrated a significant difference (p<0.05) in analyzed concentrations of E2 in soil with respect to both time and depth. The initially ascending distribution of hormones, followed by a descending pattern over the time, can be considered as a highly significant interaction between time and depth.

Monitoring the analyzed concentrations of E1 in soil samples at lower depths under the soil treatment, showed a major spatial distribution of E1 at depth 15 cm in the initial 23 days, ranging from 1.46 to 4.41 μg/kg and at a depth of 35 cm in the range of 0.41-4.96 μg/kg under soil treatment. The highest concentration of 4.413±0.77 μg/kg measured at day zero, decreased to 1.894±0.12 μg/kg one day after the irrigation at day 1 but after three days, 2.48±0.5 μg/kg was quantified which might indicate the leaching of the E2 metabolite, E1, from the surface. The E1 concentration followed an ascending pattern until day 15, reaching 3.252±0.25 μg/kg which was found to decrease after the second irrigation at day 16, and the following sampling dates. Only 0.016±0.007 μg/kg of E1 was quantified at day 31. The highest concentration of 4.958±0.35 μg/kg was measured at depth 35 cm after the first rainfall simulation which decreased to 2.845±0.27 μg/kg one day after the irrigation at day 1 and 0.405±0.19 μg/kg after three days. After day 7, a higher concentration of E1 was found as compared to day three, which increased to 0.969±0.098 μg/kg, one day after the second irrigation on day 16. 1.81±0.43 μg/kg and 1.05±0.3 μg/kg were found on days 30 and 31, indicating the potential downward leaching of the E2 metabolite, E1, from the upper depth of the soil, even one month after the application of manure. The concentration of E1 at both 15 and 35 cm was found below the detection limit on day 45 and 46.

The concentration distribution of E1 at lower depths was found to be significantly different (p<0.05) in the lysimeters receiving biochar treatments where the E1 concentration was significantly lower than the soil treatment at lower depths of the soil.
profile. The maximum concentration of E1 (1.20±0.38 μg/kg) under biochar treatment was found at depth 15 cm at day 1. The quantified concentration of E1 (0.665±0.1 μg/kg) at day 3 was 3.5 times lower than the same parameter under soil treatment which decreased to 0.113±0.06 μg/kg at day 23. After the next two irrigations on day 30 and 45, the E1 concentration was not detected in the collected soil samples. At the 35 cm depth of the soil profile, only on days 16, 23 and 31 was E1 detectable with a concentration ranging from 0.181-0.302 μg/kg. Detectable concentrations of E1 were not found at depth 65 cm under biochar treatment; however, a significantly high concentration of E1 (7.0974±0.0.272 μg/kg) was detected at day 16, decreasing to 4.30±0.31 μg/kg at day 23 and on day 31 after the third irrigation only 0.181±0.09 μg/kg was detected and on next two sampling dates, day 45 and 46, the E1 was below the detection limit.

6.5.3 Temporal stratification of E2 and E1 in leachate samples

The leachate samples were collected on day 0, 15, 30 and 45 and were analyzed in order to quantify the estrogen content of each sample. The average concentration of E2 and E1 in the leachate samples under the two treatments over time is shown in Fig 6.3. The detected concentration of hormones in the water samples was relatively lower than the hormone concentrations measured in the soil samples, which could be due to the high sorption affinity of estrogenic hormones to organic matters available in the upper soil profile. The quantified estrogen contents of water samples demonstrated a significant difference (p<0.05) between the two treatments. Initial concentration of 123.66±7.99 μg/L of E2 and 68.51±5.31 μg/L of E1 were found in leachate samples under the soil treatment after the first irrigation at day 0 which were at the highest concentrations in the period of the experiment. Only 23.81±5.16 μg/L of E2 and 12.66±4.34 μg/L) of E1 were measured in the leachate collected under biochar treatment.

After the second irrigation on day 15, lower concentrations of both estrogens (i.e. 33.68±3.43 μg/L of E1 and 61.26±10.80 μg/L of E2) were found under the soil treatment, whereas an ascending pattern was observed after the third irrigation in the concentration distribution of both estrogens under soil treatment, where 57.39±7.78 μg/L of E1 and 85.24±57 μg/L of E2 were measured. The E1 content of water samples under biochar treatment was found to be 3 times lower at day 15, 5 times lower at day 30, and 9 times
lower on day 45 after the last irrigation. 14.59±2.04 μg/L of E2 was measured on day 15 under biochar treatment which increased to 33.15±6.38 μg/L and the final concentration of E2 detected in water samples on day 45 was 13.71±0.73 μg/L under the biochar treatment. The texture and physical properties of the soil should be considered as two other governing factors affecting the fate and transport of hormones in soil. Casey et al. (2005) and Das et al. (2004) reported that higher sand content in the upper soil profile would lead to less sorption and more E2 would be dissolved in the aqueous phase as a result, causing more leaching of the hormones to lower depths. However, the longer persistence of hormones in the lower part of the soil profile could be due to less oxygen availability and less biological activity (Fan et al., 2007). Non-hydrophobic sorption interactions of E2 was hypothesized by Yu et al. (2004) by the interaction of the phenolic group of estrogens with humic acids or mineral surfaces via hydrogen and covalent bonding.

### 6.5.4 Mass balance

The quantified concentrations of E2 and E1 in the soil and water samples for all eleven sampling days were used to perform the mass balance analysis. Tables 6.1 and 6.2 present the residual hormone content in different depths of the soil profile and collected leachate samples through time for the two treatments.

The initial mass of 1816.3±5.24 μg of E2 and 3771.5±6.48 μg of E1 were introduced by applying the 2.7 L of liquid swine manure to the topsoil of each lysimeter, which dissipated in the soil profile rapidly even one day after the application. Only 10% of the preliminary mass of E2 (175.3±2.35 μg) was found in the soil surface of the lysimeters under the soil treatment after the first irrigation on day 0. One day after the first irrigation, only 25% of the initially quantified mass of E2 (45.87±1.27 μg) in the topsoil was measured. Even in the presence of biochar, a significant amount of E2 mass was lost one day after the application of manure; however, biochar was still able to retain more than 20% of the initial applied E2 mass in the topsoil indicating a significant difference resulting from the sorption ability of biochar as compared to the soil treatment. The initial E2 mass content of the soil profile under the biochar treatment was calculated to be about
478.20±2.69 μg. One day after the first irrigation, more than 50% of the initially measured E2 mass was still found in the soil surface under the biochar treatment.

The major E2 mass contribution was found in the topsoil in the presence of biochar; however, a large portion of the E2 mass was found between 15 and 45 cm in the soil profile in the lysimeters under soil treatment. The comparison of mass balance data for each depth of the soil profile demonstrates a significantly different estrogen mass distribution under the biochar treatment. This is an indication of the high retention ability of biochar to sorb the manure-borne hormones in the topsoil and decreased leaching rate of hormones. 34.56±1.45 μg of E2 was quantified in the surface under biochar treatment after the second irrigation; however, the E2 mass under the soil treatment was almost 7 times lower than the biochar treatment. The E2 mass content of soil samples collected from lower depths was significantly higher under soil treatment, whereas after 7 days, no mass of E2 was detected between 5-15 cm of soil in lysimeters receiving biochar. E2 was significantly above the detection limit until day 31 under the soil treatment. The average quantified E2 mass found in the leachate samples, collected under the soil treatment, was almost 4-fold greater overall than the biochar treatment for all four irrigation dates.

By the application of liquid swine manure, almost 3771.5±8.12 μg of E1 was introduced to the soil surface of each lysimeter. One day after the incorporation of manure and the first irrigation, only 944.73±5.46 μg of E1 was found in the topsoil of lysimeters under soil treatment indicating more than a 75% reduction of the initial E1 whereas more than 83% of E1 was still remaining in the soil surface in the presence of biochar. A dominant portion of E1 mass remained in the soil surface in the presence of biochar; however, the mass distribution of E1 was similar to E2 where the major E1 mass in lower depths was detected at depths between 15-45 cm in the soil treatment samples. 50.65±0.98 μg of E1 was measured in the 45-70 cm of soil profile under soil treatment after the second irrigation while only 1.50±0.43 μg of E1 was detected in the surface. Biochar was able to hold 283.24±0.88 μg in the surface and only 0.78±0.012 μg of E1 was detected between 45-75 cm. 4-fold greater mass of E1 was quantified in the leachate samples collected under soil treatment, as compared to biochar, in all four irrigations. After the last
irrigation on day 45, the E1 content of water samples receiving biochar was 20 times lower than E1 recovered in the water samples collected under soil treatment. The different mass distribution of estrogens in the presence of biochar can be explained by the potential capacity of biochar to provide heterogeneous sorption sites consisting of pre-existing meso- and micropore cavities with different pore sizes and pore surfaces, which results in the heterogeneity of nonspecific sorption-site energies where van der Waals forces play the dominant role as the solute-sorbent interactions (Xing et al., 1996) indicating a better perspective for remediation by biochar as a soil amendment.

6.5.5 Degradation kinetics of estrogens under the field condition

The degradation kinetics of both E2 and its metabolite, E1, in the two treatments were determined based on the first-order decay (Johnson, 2001) with the governing model as follows:

\[
\frac{dC_t}{dt} = -kt \quad (3)
\]

Integrating Eq. (3) gives

\[
C_t = C_o e^{-kt} \quad (4)
\]

Where \(C_t\) is the residual hormone concentration at time (t), \(C_o\) is the initial concentration, and \(k\) is the degradation rate constant. The natural logarithm of mass/initial mass of each hormone versus time was plotted where the slope of the linear graph represented the degradation rate constant (k), which was used to calculate the half-lives of estrogens in soil.
Fig 6.1 Concentration of E2 in the soil and soil-biochar treatments at four sampling depth of lysimeter receiving Swine manure (log scale used for Y axis plotting)
Fig 6.2 Concentration of E1 in the soil and soil-biochar treatments at four sampling depth of lysimeter receiving Swine manure (log scale used for Y axis plotting)
Fig 6.3 Concentration of E2 and E1 in lysimeter’s leachate samples at four sampling dates (Days 0, 15, 35 and 45) under two treatments.
Table 6.1 Mass balance profile of E2 (μg) in the lysimeter soil profile and leachate samples over the 45 day period in swine manure

<table>
<thead>
<tr>
<th>Soil profile (cm)</th>
<th>Soil treatment</th>
<th>0-5</th>
<th>5-15</th>
<th>15-45</th>
<th>45-70</th>
<th>Cum. leachate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>175.30</td>
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<td>42.20</td>
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<td>**0.86</td>
</tr>
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<td>3</td>
<td></td>
<td>16.66</td>
<td>9.04</td>
<td>2.35</td>
<td></td>
<td>**</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>5.93</td>
<td>3.93</td>
<td>6.22</td>
<td></td>
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<td>15</td>
<td></td>
<td>4.81</td>
<td>1.90</td>
<td>5.33</td>
<td></td>
<td>**0.43</td>
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<td>2.21</td>
<td>5.51</td>
<td>18.31</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td></td>
<td>5.25</td>
<td>1.61</td>
<td>2.99</td>
<td>5.51</td>
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<td>30</td>
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<td>9.9</td>
<td>4.83</td>
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<td>11.23</td>
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<td>45</td>
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<td>**</td>
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<td>**</td>
<td>**0.60</td>
</tr>
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<td>46</td>
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<table>
<thead>
<tr>
<th>Soil profile (cm)</th>
<th>Soil-Biochar treatment</th>
<th>0-5</th>
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<th>15-45</th>
<th>45-70</th>
<th>Cum. leachate</th>
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</tr>
<tr>
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</tr>
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<td>8.98</td>
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<td>0</td>
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<td>0.23</td>
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<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Mass of E1 (μg) in total collected volume of leachate samples after each irrigation

**Below the detection limit of E1 in analytical process

a Standard deviation
Table 6. Mass balance profile of E1 (μg) in the lysimeter soil profile and leachate samples over the 45 day period in swine manure

<table>
<thead>
<tr>
<th>Soil treatment</th>
<th>0-5</th>
<th>5-15</th>
<th>15-45</th>
<th>45-70</th>
<th>Cum. leachate</th>
<th>Total</th>
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<tbody>
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<td>Soil profile (cm)</td>
<td>Day</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>944.73</td>
<td>1006.58 ± 0.8a</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>114.85</td>
<td>148.43 ± 0.45</td>
</tr>
<tr>
<td>3</td>
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<td></td>
<td></td>
<td>30.52</td>
<td>38.43 ± 0.66</td>
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<tr>
<td>7</td>
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<td></td>
<td></td>
<td></td>
<td>16.92</td>
<td>30.56 ± 0.48</td>
</tr>
<tr>
<td>15</td>
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<td></td>
<td></td>
<td></td>
<td>3.01</td>
<td>14.18 ± 0.14</td>
</tr>
<tr>
<td>16</td>
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<td></td>
<td></td>
<td>1.15</td>
<td>60.47 ± 0.97</td>
</tr>
<tr>
<td>23</td>
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<td></td>
<td></td>
<td>1.72</td>
<td>40.39 ± 0.74</td>
</tr>
<tr>
<td>30</td>
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<td></td>
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<td></td>
<td>0.57</td>
<td>18 ± 0.88</td>
</tr>
<tr>
<td>31</td>
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<td></td>
<td></td>
<td>0.06</td>
<td>13.05 ± 0.54</td>
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<tr>
<td>45</td>
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<td></td>
<td></td>
<td></td>
<td>** **</td>
<td>0.4 ± 0.012</td>
</tr>
<tr>
<td>46</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>** **</td>
<td>** **</td>
</tr>
</tbody>
</table>

Soil-Biochar treatment

| Soil profile (cm) | Day | | | | | | |
|------------------|-----| | | | | | | |
| 0                |     | | | | 3158.8 | 3161.93 ± 0.67a |
| 1                |     | | | | 2719.7 | 2723.35 ± 0.26 |
| 3                |     | | | | 1257.4 | 1258.58 ± 0.53 |
| 7                |     | | | | 473.64 | 474.07 ± 0.22 |
| 15               |     | | | | 345.57 | 345.83 ± 0.11 |
| 16               |     | | | | 283.24 | 285.17 ± 0.66 |
| 23               |     | | | | 138.58 | 141.19 ± 0.13 |
| 30               |     | | | | 182.87 | 182.97 ± 0.89 |
| 31               |     | | | | 42.77  | 44.77 ± 0.45 |
| 45               |     | | | | 0      | 0.02 ± 0.0 |
| 46               |     | | | | 0      | 0 |

*Mass of E1 (μg) in total collected volume of leachate samples after each irrigation

**Below the detection limit of E1 in analytical process

a Standard deviation
The model-fitting parameters are presented in Table 6.3. The first-order rate degradation model perfectly explains the degradation of estrogenic hormones in soil under field conditions. The degradation rate constants of E2 under biochar and soil treatment, were respectively 0.0017 h$^{-1}$ and 0.0024 h$^{-1}$, which is acceptable based on a wide range of reported first-order degradation/transformation rate constants of E2 (0.0006 h$^{-1}$) (Fan et al., 2007) and 0.252 h$^{-1}$ (Layton et al., 2000). The half-life of E2 was found to be different under the two treatments, where the half-life of E2 was determined 15 days in the lysimeters receiving biochar; however, the E2 reached half of its concentration after 11 days under the soil treatment. The half-life of E1 was calculated 7 days under the biochar treatment whereas the half-life of E1 was 3 days under the soil treatment, indicating that estrogenic hormones are moderately persistent in the natural matrix in the presence of biochar under field conditions.

Several studies reported a short half-life for estrogens in soil and water media; for example, Colucci et al. (2001) and Jacobsen et al. (2005) reported the degradation of E2 within 24 h which was accompanied by an accumulation of E1, which subsequently degraded within 72 h. Xuan et al. (2008) mentioned the influential effect of moisture content, redox conditions and temperature on the degradation of both hormones. They reported the degradation dependency on soil temperature where they found at 15 °C, the rate constant (k’) and the half-life were 0.141±0.008 day$^{-1}$ and 4.9 days, respectively. When the temperature was increased to 25 °C, the rate constant increased to 0.750 day$^{-1}$, which is 4.3 times higher than at 15 °C and correspondingly, the half-life was reduced to 0.92 days.

Table 6.3 Linear regression and half-lives of E2 and E1 under the two treatments

<table>
<thead>
<tr>
<th>Biochar treatment</th>
<th>Regression slope (k)</th>
<th>Coefficient of determination ($r^2$)</th>
<th>Half-life (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2</td>
<td>0.0404</td>
<td>0.86</td>
<td>15</td>
</tr>
<tr>
<td>E1</td>
<td>0.0866</td>
<td>0.85</td>
<td>7</td>
</tr>
<tr>
<td>Soil treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>0.057</td>
<td>0.88</td>
<td>11</td>
</tr>
<tr>
<td>E1</td>
<td>0.2135</td>
<td>0.90</td>
<td>3</td>
</tr>
</tbody>
</table>
Another major process influencing the stratification of hormones in soil and water media is the rapid biodegradation of estrogens. Being a carbon source for cell growth through metabolization (Donova, 2007; Korom, 1992), steroid hormones are considered as a source of energy for bacteria in a redox (reduction/oxidation) reaction. The main bacterial transformation processors of steroid hormones in environmental media are Mycobacterium, Arthrobacter, Bacillus, PSeudomonas, Escherichia and Micrococcus (Fernandes et al., 2003; Jenkins et al., 2004; Malaviya and Gomes, 2008). Danielsson and Zhang (1996) indicated that the biotransformation of estrogens takes place until log $K_{ow}$ reaches 3.0-3.5 but at higher log $K_{ow}$ values, sorption and solid-phase partitioning would be the dominant procedure governing the behavior of estrogens. Fan et al. (2007) found that biological, rather than other physical-chemical processes play a more significant role.
on the fate and degradation of these hormones while investigating the persistence of E2 and E1 in agricultural soil. In their study, they found higher extractability and bioavailability of estrogens in soils with low organic content and this could lead to higher rates of hormone degradation and a higher risk of runoff and leaching to surface and subsurface water resources. In the investigation of degradation of estrogens in the presence of various organic matrices, including animal wastes and biosolids under aerobic conditions, Jacobsen et al. (2005) indicated that the major degradation of hormones in soil is caused by microbial degradation and is influenced by the temperature and pH of the media where the first degradation rate of E2 was 0.01d\(^{-1}\). The redox conditions (aerobic vs. anaerobic) can significantly influence the degradation of hormones whereas Kjær et al. (2007) found that the incorporation of manure containing sex hormones in the topsoil would result in soil conditions potentially remaining anoxic for a longer period of time, therefore, would retard the degradation of hormones with a greater chance of bioavailability. However in this study, the effect of redox conditions was not evaluated.

6.5.6 Multi-action roles of biochar as soil amendment

The chemical and structural properties of the biochar-soil matrix (e.g. the particle size distribution, specific surface area and total porosity) and the physic-chemical properties of the organic contaminants (e.g. hydrophobicity or charge characteristics) have been highlighted as the two dominant factors governing their sorption behavior (Kookana, 2010). Different feedstock and the conditions under which the thermo-decomposition of biomass takes place (e.g. heating rate and temperature) will affect the carbon content and the degree of aromatic condensation of the biochars (Sarmah et al., 2010), which are considered as major variables influencing the feasible remediation capacity of different types of biochars for organic contaminants in the soil-biochar media.

6.5.7 Sorption mechanism of biochar

The structural characteristic of biochar is a fundamental factor in determining its sorption capacity. The soil particle size distribution and its organic matter content are the other two dominant factors controlling the sorption-desorption isotherms of organic chemicals.
The carbonization of biochar is a fundamental structural property affecting its sorption behavior. The heterogeneous surface of biochar is due to the carbonized and non-carbonized fractions of biochar, known as two major composition structures (Cao et al., 2009; Chen et al., 2008). The carbonized and non-carbonized fractions of biochar apply different functions on the sorption isotherms of hormones (linear vs. non-linear sorption behavior) in the presence of biochar where the carbonized fraction of biochar performs as the glassy domain function (i.e. the nonlinear sorption isotherms) and the non-carbonized fraction represents the rubbery domain function (i.e. linear sorption isotherms). The rubbery phase performance of the soft carbon domain and the similar glassy phase performance of the hard carbon domain, known as the two predominant domains in the heterogeneous sorbents (e.g. soil organic matters) with different sorption function, have been reported (Weber Jr and Huang, 1996; Xia and Ball, 1999; Xia and Pignatello, 2001; Xing et al., 1996).

The partition sorbent behavior of the rubbery state and the free voids are due to the rigidity of the glass state semi-permanent nano scale porous zone which are considered as adsorption sites with an internal high surface for soil organic matter. Zheng et al. (2010) attributed the sorption of the hydrophobic compounds from an aqueous solution to the highly hydrophobic surface of biochar; contact with water would modify the hydrophobicity behavior which distinguishes biochar from other carbonaceous polymeric substances (Chun et al., 2004). The structure of the biochar comprises of macromolecules particles with flexible pores (Engel and Macko, 1993; Killops et al., 1993) consisting of aliphatic, alicyclic, aromatic and hetero-aromatic backbones cross-linked by metal ions or covalent bonds (Aiken et al., 1985; Hayes et al., 1989) with abundant mobile nuclei in their cross-linked structures and humic substances (Xia and Pignatello, 2001) which can adsorb small molecules into their matrix.

The preliminary phase of sorption of organic chemicals in the presence of biochar is a function of the gradual intra-particle diffusion mechanics (Pan et al., 2008; Valderrama et al., 2008; Zheng et al., 2010). Zhu et al. (2005) reported the assisted sorption of aromatic hydrocarbons to a wood char by π-electron interactions followed by a pore-
filling mechanism (Nguyen et al., 2007). The disparity of structural properties of different biochars is due to the variety of feedstocks and the quality of the organic materials. Therefore, the sorption behavior of organic contaminants in biochar-amended soil can be significantly influenced by the specific surface area and cation exchange capacity, lower ash content and volatile matter, higher organic carbon content, different organic materials, particle size and the pyrolysis conditions. The simultaneous presence of different organic chemicals in the soil media may impact their sorption behavior in the presence of organic sorbents like biochar and the comparative sorption between these contaminants should be considered as an important factor when evaluating the environmental remediation ability of biochar. In the case of naturally manure borne hormones, the coexistence of veterinary antibiotics with estrogenic hormones in animal waste and animal manure-fertilized agricultural soil may greatly reduce the biodegradation of estrogens in the environment by affecting microorganisms at a very low toxic dose. Therefore, the influence of the coexistence of other manure-borne organic contaminants should be considered in the evaluations of the fate and transport of estrogenic hormones in the environment.

6.6 Conclusion

A 45-day field scale lysimeter study was conducted in order to investigate the retention ability of slow pyrolysis biochar to reduce the manure-borne hormonal pollution in soil and water under field conditions. The main goal of the study was to investigate the fate and transport of two female sex hormones, E2 and E1, in soil amended with 1% slow pyrolysis biochar, receiving liquid swine manure. The highly effective retention capability of biochar as a soil-amendment for reducing the manure-borne hormonal pollution in soil and water was confirmed by the observed significant difference between the temporal–spatial distribution concentration of E2 and E1 in soil under the two treatments, soil and soil-biochar, at four sampling depths including the surface, 15, 35 and 65 cm, over 46 days. The results of this study demonstrated that the higher sorption affinity and stronger desorption resistance of biochar should be considered as factors regulating the aqueous concentration of these contaminants, and as a result, affect leaching, the environmental bioavailability and degradation of these hormones. This
research confirmed the potency of biochar not only as an organic soil amendment for improving the soil quality and crop production but also as a novel remediation technique for better sustainable conservation of water resources.
PREFACE TO CHAPTER 7

In the three previous chapters, the significant of biochar was observed for reducing the manure borne female sex contamination in soil and water samples in lysimeters. Estrogens are the dominant naturally manure-borne female sex hormones.

In this chapter, the influence of biotic and abiotic transformation mechanisms, including biodegradation, photo-degradation and chemical transformation of three female sex hormones where evaluated in the soil where the effect of biochar was also investigated on these mechanisms.

To date, this is the first study investigating the influence of biochar on degradation behavior of female sex hormones. This research will help us understand different mechanisms affecting the environmental behavior of hormones and the role of biochar in these processes.

Research papers based on the chapter:

S.Alizadeh, F.Gobbi, S.O.Prasher. Influence of Biodegradation and Chemical Transformation and Photo-degradation on the Fate and Transport of Female Sex Hormones in the Presence of Slow Pyrolysis of Biochar
Chapter 7
Biodegradation, Chemical Transformation and Photo-degradation of Female Sex Hormones in the Presence of Slow-Pyrolysis Biochar

7.1 Abstract

The influence of biodegradation, chemical transformation and photo-degradation mechanisms on the persistence and dissipation of three natural female sex hormones, 17β-estradiol (E2), estrone (E1) and progesterone (P) were investigated. The effect of slow pyrolysis biochar as a soil amendment on each degradation mechanism was also evaluated. No significant effect of photo-degradation on the dissipation of estrogens and progesterone in soil and soil-biochar media was observed. However biodegradation and chemical transformation demonstrated a significant effect on the persistence of these hormones; E2 was dominantly degraded through biodegradation; however, progesterone and E1 were mainly decomposed by chemical transformation. The half-life all three hormones was less that 3 days under laboratory conditions. No significant difference was observed on the degradation behavior of hormones in the presence of biochar.

Keyword : Sex hormones, degradation, half-life, biochar

7.2 Introduction

Over the last decade, several cases of biological abnormalities including sexual, developmental and reproductive disorders have been reported in the aquatic environment. These abnormalities are linked to the occurrence of a new paramount class of emerging contaminants with endocrine disrupting properties. They are classified as Endocrine Disrupting Chemicals (EDCs) and they have the potential to modulate the functions of the endocrine system by mimicking, counteracting, altering or interfering with the metabolism and biosynthesis of endogenous hormones (Colucci et al., 2001). Natural and synthetic steroid sex hormones have been categorized as the dominant class of EDCs due to their detrimental and carcinogenetic effects at extremely low environmental concentrations. Recently, an increasing number of female endocrine disorders such as polycystic ovary syndrome (PCOS) and breast cancer as well as numerous cases of
prostate and testicular cancers have been linked to the environmental presence of these toxic hormones. For both male and female steroid sex hormones, the major concern is the potential high-risk female sex hormones, 17β-estradiol (E2) and its primary metabolites, estrone (E1) and progesterone (P); this is due to their high biotransformation ability and environmental stability.

The reproductive biology alteration of wild fathead minnows (Pimephales promelas) and rainbow trout at aquatic E2 concentrations as low as 1-10 ng/L and 25-50 ng/L of E1 (Fang et al., 2003; Young and Borch, 2009) or the feminization of male fish or the masculinization of female fish (Vajda et al., 2011) were reported recently as an indication of hormonal contamination in surface water resources. Blazer et al. (2007) have reported the intersexuality in fish exposed downstream of wastewater treatment plants and drainage effluents. The potential effect of these sex hormones on, not only, the primary sexual traits and reproduction functions but also the secondary sexual traits, which can affect mating dynamics, have been reported recently by Partridge et al. (2010). The application of biosolids, animal manure and wastewater plant (WTPs) discharges, generated by intensive agriculture and expanding anthropogenic activities such as concentrated animal feeding operations, have been recognized as the major source of environmental exposure of these chronic chemicals. The environmental occurrence and behavior of these hydrophobic, high-toxicity-at-low-concentration hormonal compounds would be dominantly influenced by various physical, chemical and biotransformation processes such as sorption and desorption in the soil media (Ying and Kookana, 2009) and biodegradation or photo-degradation (Chowdhury et al., 2010).

Recently, several studies have investigated the sorption-desorption behavior of these hormones in soil media. Rapid sorption kinetics with relatively large organic-carbon-normalized sorption coefficients of estrogens (log \( K_{OC} \sim 2.8\text{-}3.8 \)) and low aqueous solubility have been reported (Lee et al., 2003). The rapid degradation of estrogens and progesterone with short half-lives have been reported (Combalbert and Hernandez-Raquet, 2010; Hansen et al., 2011). Jacobsen et al. (2005) reported the degradation of E2 within 24 h, which was accompanied by an accumulation of E1 which subsequently degraded within 72 h. However, detailed knowledge is limited on the topic of the biotic
and abiotic transformation of hormones in soil and water media. The physical and chemical parameters affecting the persistence and dissipation of these female hormones through these processes is not well-understood. The inadequate knowledge regarding the remediation of these sex hormones in the soil matrix and aquatic media has resulted in an important concern about the hazardous environmental availability of steroid sex hormones.

Biochar is an organic soil-amendment derived from biological matter, known as the byproducts of pyrolysis of biomass; it can be added to topsoil to engineer the natural physical and chemical processes influencing the fate and transport of sex hormones. It has been proposed as a feasible remediation technique in order to reduce the risk of hormonal pollution in the aquatic environment with the minimum pretreatment requirement. Biochar can be applied directly to the soil and provides a convenient way to dispose of organic waste with the ability to bind pollutants and reduce their bioavailability while promoting plant growth and stimulating ecological restoration. In this study, the influence of biotic and abiotic transformation including microbial, chemical and photo-degradation of three female sex hormones, E2, E1, and P was investigated under two different media: soil and soil amended with 1% slow pyrolysis biochar. All three degradation patterns were studied under constant temperature and moisture constant and light intensity in order to reduce possible sources of error.

7.3 Materials and Methods

The detailed methodology information including, the soil and biochar physical and chemical properties, extraction of hormones from soil, hormonal content analysis are presented in Chapter 4, section 4.3 and Chapter 5, section 5.3.1.

7.4 Overall Methodology

In order to investigate the influence of the biotic and abiotic degradation and transformation of hormones on their fate and transport in soil and water media, two separate laboratory degradation experiments were conducted under simulated field conditions using the modified method used by Xuan et al. (2008). The main goal of the
first experiment was to determine which degradation mechanism (biotic vs. abiotic), including photo-degradation, chemical and biodegradation, significantly influence the environmental behavior of manure-borne steroid hormones. The second experiment was conducted in order to determine the degradation kinetics of all three hormones under each degradation pattern. This study was also focused on investigating the influence of slow pyrolysis biochar, as a soil amendment, on the degradation kinetics of steroid hormones in soil.

7.4.1 Photo-degradation, chemical and biodegradation of steroid sex hormones in the presence of slow-pyrolysis biochar

This study was conducted to assess the role of different degradation mechanisms, including photo-degradation and microbial degradation, on the dissipation and half-life of naturally manure-borne estrogens and progesterone and the influence of biochar amendment, as environmental remediation of hormones, on these processes. Soil samples were prepared under four different treatments, including the non-sterilized soil, non-sterilized soil amended with 1% non-sterilized slow pyrolysis biochar and sterile soil and sterile soil amended with 1% sterile biochar, including three replicates per treatment. The soil was air-dried for 24 hours and both sterile soil and biochar were prepared by autoclaving the materials at 121 °C for 1 hour. For each treatment, 5 g of soil was weighed in a sterilized petri-dish and was initially spiked with 1 mL of 5 mg L⁻¹ concentration stock solution containing all three hormones. The initial stock solution of each hormone was prepared in methanol whereas the main spiking standard solution of hormones was prepared in Milli-Q water.

In order to evaluate the effect of photo-degradation, all samples were prepared with sterile soil and biochar and then they were covered with aluminum foil, in order to block the light exposure to the soil surface. In order to study the degradation pattern of hormones in a real case scenario, we tried to simulate the field conditions based on the weather and sunlight intensity data collected during our field-scale lysimeters study of the fate and transport of manure-borne hormones in soil. After preparation of samples, all samples were kept in the plant growth chamber with a controlled average temperature of 25°C and controlled humidity of 70%, under constant 24-hour incandescent and
fluorescent light, with the intensity of 200 (μmoles /m²/s). Each batch of samples under each treatment was analyzed after 0, 48 and 120 hours in order to quantify the residual hormone content of the soil samples.

7.4.2 Study to determine the effect of slow pyrolysis biochar on biodegradation and chemical degradation kinetics of steroid sex hormones in soil

The biodegradation of three steroid hormones were investigated over 5 days under simulated field conditions. The main objectives of this study were to evaluate the effect of biochar amendment on the degradation pattern of manure-borne steroid hormones. The biodegradation of each hormone was studied separately. Soil samples were prepared under four different treatments including non-sterile soil, non-sterile soil amended with 1% slow pyrolysis biochar, sterile soil and sterile soil amended with 1% sterile biochar. All soil samples under each treatment were spiked with 1 mL of each hormone standard solution with 5 mg L⁻¹ concentration separately. Similar to the first experiment, the biodegradation experiment was conducted under controlled average temperatures of 25°C and a controlled humidity of 70%, under constant 24-hour incandescent and fluorescent light with the intensity of 200 (μmoles/m²/s) in the growth chamber. In order to find the degradation rate and half-life of each hormone, each batch of samples was analyzed after 0, 48 and 120 hours to quantify the hormonal content of the soil samples. The degradation kinetics of all three hormones, under each treatment, were calculated using the first-order decay model (Johnson, 2001). The analysis of non-sterile soil samples helped us to determine the overall microbial and chemical degradation of each hormone where the chemical transformation of each hormone was obtained by analysis of residual hormones in sterile soil samples at two sampling times (48 and 120 hours). Finally, the difference between the degradation behavior of each hormone under non-sterile and sterile soil samples was used to find the degradation kinetics and dominant mechanisms influencing the fate of these hormones in soil.

7.4.3 Kinetic modeling

The degradation kinetics of three hormones degradation in the soil matrix can be explained by the first-order degradation kinetics where the degradation rate of the each
hormone is a function of the concentration of the remaining compound in the soil over time (Xuan et al., 2008).

7.5 Results

The amount of degradation of each hormone was calculated based on the initial spiked concentration and the analytically quantified concentration of each hormone under each treatment in order to assess the influence of photo-degradation, microbial and chemical degradation on the dissipation of steroid hormones. The analyzed results under the treatments amended with 1% slow pyrolysis biochar were used to evaluate the role of biochar in each degradation mechanism. The degradation of each hormone (%) was calculated over the two analysis times (i.e. 48 and 120 hours) for each individual treatment and it is given in Fig 7.1.

7.5.1 Photo-degradation

In order to quantify the influence of photo-degradation on the dissipation behavior of steroid hormones, the percentage of degradation of hormones were compared under exposed sterile soil and covered sterile soil treatments. It was observed that only 8.5 % of the initial E2 was photo-degraded after 48 hours. There was no significant difference between the degradation of hormones in the samples exposed to light and the covered samples, thus, indicating the insignificant influence of photo-degradation. No significant difference was observed between the amounts of hormones degraded in the presence of biochar, even over time. It was also observed that amending the sterilized biochar to the sterile soil samples did not significantly influence the photo-degradation pattern of E2. Almost 37% and 41% of E1 was degraded in sterile soil exposed to light after 48 and 120 hours, respectively, whereas only 10% of E1 was degraded after 48 hours in covered sterile soil samples; this increased to 13% at 120 hours. The photo-degradation rate of E1 in the uncovered sterile mixture of soil and biochar was smaller than the samples under cover, demonstrating no significant influence of radiation on the degradation behavior of E1. No statistically significant difference (P<0.05) was observed between the amount of degraded E1 in sterile soil and sterile soil amended with biochar, when both were exposed to light. No significant difference was found between the calculated
progesterone degradation of covered and un-covered sterile soil samples. Less than 8% of degradation was exhibited as a result of exposure to sun-light radiation. Unless photo-degradation of progesterone takes place in soil media, it did not significantly influence the stratification of progesterone in soil media. Comparing the amount of degraded progesterone in the mixture of sterile soil-biochar in both the covered and un-covered samples demonstrated similar observations as in the sterile soil treatment. The photo-degradation of progesterone in sterile soil amended with 1% sterile biochar was found to be 10% less than the sterile soil without biochar treatment, after 48 hours. After 120 hours, only 38.2% of progesterone was photo-degraded in the presence of biochar; however, in sterile soil samples, the degradation rate was found to be more than 58%. The observed difference in the presence of biochar was not statistically different.

7.5.2 Microbial and chemical degradation

The comparison of degradation percentage of each hormone under the soil and sterile soil treatment was used to determine the effect of simultaneous microbial and chemical degradation. The leading role of microbial and chemical degradation on the fate of progesterone was observed over time. Based on the measured concentration of E2 under soil treatment, almost 61% of the hormone was degraded after 48 hours; however, more than 75% of E2 was quantified in the sterile soil samples with the same sampling time, indicating more than 38% of microbial degradation and chemical transformation of E2. The degradation behavior of E2 was also investigated over time. A decrease in degradation potential of E2 was observed over time as a smaller degradation percentage of E2 was quantified after 120 hours in soil samples under the soil treatments; this might be due to the loss of microbial species or their interest for further degradation. Almost 60% of the initial spiked progesterone concentration was degraded after 48 hours which increased to 73% after 120 hours in non-sterile soil samples. Based on the comparison of initial and measured concentrations of progesterone after 48 hours and 120 hours in sterile soil samples, only 37 % and 57.8% of progesterone was degraded. More than 23% of initial E1 was degraded in sterile soil samples after 48 hours indicating the dominant role of chemical transformation in the degradation behavior of E1. The preliminary results obtained from the analysis of the first degradation experiment samples
demonstrated the insignificant effect of photo-degradation on the dissipation of estrogens and progesterone in soil and soil-biochar media where biodegradation and chemical transformation demonstrated a critical effect on the persistence and dissipation of these hormones. For both E2 and P, the photo-degradation rate was less than 10% whereas E1 demonstrated the higher potential for photo-degradation. However for all three hormones, the effect of photo degradation was not statistically significant.

7.5.3 Degradation kinetics of female sex hormones

After determining the non-significant effect of photo-degradation, the microbial degradation and chemical transformation of the three hormones were investigated individually.

7.5.3.1 Microbial and chemical degradation behavior of E2

The quantified concentration of E2 in non-sterile soil samples demonstrated more than 94% degradation of E2 after 48 hours where, after 120 hours, less than 4% of the initial E2 was detectable. The analysis of the sterile soil samples demonstrated almost 43% of chemical transformation and 52.5% microbial degradation influencing the persistence of E2 in soil. The half-life of E2 in the non-sterile soil was found 1.04 day at 25 °C which is consistence with Colucci et al. (2001). However in sterile soil samples, E2 was found to be more persistence with a half-life of 4.05 days. The analyzed hormonal content of soil samples under both sterile and non-sterile soil-biochar treatments demonstrated similar results to soil treatment data, where almost 50% of E2 was chemically transformed with 40% of microbial degradation in biochar amended media. Biochar reduced the degradation percentage of E2 in both sterile and non-sterile media; nevertheless, the difference was not statistically significant.
Fig 7.1. Degradation behavior three female sex hormones under sterile and non-sterile treatments

**17β-estradiol**

- Soil
- Sterile soil
- Soil + Bio char
- Sterile soil: Bio char
- Sterile soil covered
- Sterile soil: Bio char: covered

<table>
<thead>
<tr>
<th>Treatment</th>
<th>48 hrs</th>
<th>120 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>60.3%</td>
<td>68.5%</td>
</tr>
<tr>
<td>Sterile soil</td>
<td>26.6%</td>
<td>32.4%</td>
</tr>
<tr>
<td>Soil + Bio char</td>
<td>15.3%</td>
<td>14.6%</td>
</tr>
<tr>
<td>Sterile soil: Bio char</td>
<td>52.2%</td>
<td>36.0%</td>
</tr>
<tr>
<td>Sterile soil covered</td>
<td>32.1%</td>
<td>19.6%</td>
</tr>
<tr>
<td>Sterile soil: Bio char:</td>
<td>55.4%</td>
<td>37.0%</td>
</tr>
</tbody>
</table>

**Estrone**

- Soil
- Sterile soil
- Soil + Bio char
- Sterile soil: Bio char
- Sterile soil covered
- Sterile soil: Bio char: covered

<table>
<thead>
<tr>
<th>Treatment</th>
<th>48 hrs</th>
<th>120 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>35.2%</td>
<td>30.8%</td>
</tr>
<tr>
<td>Sterile soil</td>
<td>23.6%</td>
<td>18.4%</td>
</tr>
<tr>
<td>Soil + Bio char</td>
<td>13.4%</td>
<td>17.2%</td>
</tr>
<tr>
<td>Sterile soil: Bio char</td>
<td>42.5%</td>
<td>36.4%</td>
</tr>
<tr>
<td>Sterile soil covered</td>
<td>18.7%</td>
<td>18.4%</td>
</tr>
<tr>
<td>Sterile soil: Bio char:</td>
<td>50.7%</td>
<td>38.0%</td>
</tr>
</tbody>
</table>

**Progesterone**

- Soil
- Sterile soil
- Soil + Bio char
- Sterile soil: Bio char
- Sterile soil covered
- Sterile soil: Bio char: covered

<table>
<thead>
<tr>
<th>Treatment</th>
<th>48 hrs</th>
<th>120 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>59.4%</td>
<td>38.2%</td>
</tr>
<tr>
<td>Sterile soil</td>
<td>37.2%</td>
<td>46.1%</td>
</tr>
<tr>
<td>Soil + Bio char</td>
<td>32.4%</td>
<td>38.2%</td>
</tr>
<tr>
<td>Sterile soil: Bio char</td>
<td>27.7%</td>
<td>27.7%</td>
</tr>
<tr>
<td>Sterile soil covered</td>
<td>19.0%</td>
<td>19.0%</td>
</tr>
<tr>
<td>Sterile soil: Bio char:</td>
<td>52.4%</td>
<td>52.4%</td>
</tr>
</tbody>
</table>
Table 7.1 Degradation kinetics of three female sex hormones

<table>
<thead>
<tr>
<th>Degradation kinetics of E2</th>
<th>Degradation rate constant (k)</th>
<th>Half-life ($t_{1/2}$)-day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-sterilized soil</td>
<td>0.607</td>
<td>1.04</td>
</tr>
<tr>
<td>Sterilized soil</td>
<td>0.171</td>
<td>4.05</td>
</tr>
<tr>
<td>Non-sterilized soil + 1%  biochar</td>
<td>0.459</td>
<td>1.51</td>
</tr>
<tr>
<td>Sterilized soil + 1% biochar</td>
<td>0.178</td>
<td>3.90</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Degradation kinetics of E1</th>
<th>Degradation rate constant (k)</th>
<th>Half-life ($t_{1/2}$)-day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-sterilized soil</td>
<td>0.099</td>
<td>2.69</td>
</tr>
<tr>
<td>Sterilized soil</td>
<td>0.258</td>
<td>7</td>
</tr>
<tr>
<td>Non-sterilized soil + 1%  biochar</td>
<td>0.167</td>
<td>4.15</td>
</tr>
<tr>
<td>Sterilized soil + 1% biochar</td>
<td>0.146</td>
<td>4.76</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Degradation kinetics of P</th>
<th>Degradation rate constant (k)</th>
<th>Half-life ($t_{1/2}$)-day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-sterilized soil</td>
<td>0.172</td>
<td>2.81</td>
</tr>
<tr>
<td>Sterilized soil</td>
<td>0.247</td>
<td>4.03</td>
</tr>
<tr>
<td>Non-sterilized soil + 1%  biochar</td>
<td>0.233</td>
<td>2.97</td>
</tr>
<tr>
<td>Sterilized soil + 1% biochar</td>
<td>0.149</td>
<td>4.65</td>
</tr>
</tbody>
</table>

7.5.3.2 Microbial and chemical degradation behavior of E1

E1 demonstrated the slowest degradation rate of all three hormones. Only 55% of E1 was degraded in non-sterile soil after 48 hours. The chemical transformation was as dominant a degradation mechanism for E1 with rate of 37.3% and 41% after 48 and 120 hours, respectively. The half-life of E1 in non-sterile and sterile soil was found at 2.6 and 7 days. No significant effect of biochar on both microbial and chemical degradation of E1 was observed.

7.5.3.3 Microbial and chemical degradation behavior of progesterone

Based on the progesterone content of sterile soil samples, almost 48% of the initial progesterone was chemically transformed after 48 hours which increased to 59% after 120 hours. Only 20% of the initial progesterone was degraded by microbial species in the first 48 hours which decreased to 13.8% over the next 36 hours. The significant different between the degradation rate of progesterone was only observed between soil and biochar-amended soil samples under both sterile and non-sterile soil samples for the first 48 hours; however, the final degraded amount of hormones was found not to be statistically significant in the presence of biochar after 120 hours. The dominant chemical transformation of progesterone in the presence of sterile biochar was also observed with
rate of 27% and 52.6% after 48 and 120 hours, respectively. The calculated half-life of progesterone in non-sterile soil and soil-biochar media were calculated 2.8 and 2.9 days; however, in the sterile soil, progesterone was more persistent with a half-life of 4 days.

7.6 Discussion

The determined half-life of E2 and E1 in this study was consistent with the reported 1-7 day half-lives for estrogens at 20°C (Jürgens et al., 2002). Several studies reported a short half-life for estrogens in soil and water media; for example, Colucci et al. (2001) reported the degradation of E2 within 24 h which was accompanied by an accumulation of E1 which subsequently degraded within 72 h. The biotransformation of estrogens takes place until log $K_{ow}$ reaches 3.0-3.5 but at higher log $K_{ow}$ values, sorption and solid phase partitioning would be the dominant procedure governing the behavior of estrogens (Danielsson and Zhang, 1996). The biodegradation of estrogens can be explained by the potential capability of microorganisms to transform these hormones in different ways. For instance, the biodegradation of E2 to E1 takes place over a reduction/Oxidation reaction. Being a carbon source for cell growth through metabolization (Donova, 2007; Korom, 1992), these hormones are considered a source of energy for bacteria in a redox (reduction/oxidation) reaction; since they need an electron donor as the energy source for the oxidizing E2 to E1 and an external electron acceptor during the respiration process to provide the available oxygen under aerobic conditions. Biological rather than other physical-chemical processes play a more significant role on the fate and degradation of E2 as reported by Fan et al. (2007); however, a significant portion of E1 and P was chemically transformed.

The main bacterial transformation processors of estrogens and progesterone in natural soil are Mycobacterium, Arthrobacter, Bacillus, Pseudomonas, Escherichia and Micrococcus, Bacillus and Nectria (Fernandes et al., 2003; Jenkins et al., 2004; Malaviya and Gomes, 2008). These bacteria are categorized as gram-positive bacteria, which influence the degradation process by several possible patterns; this is accomplished mainly by introducing hydroxyl groups at numerous positions along the steroid skeleton and sequential hydroxylations along carbons 22-27 of the sterol side chain (Young and Borch, 2009). Double bond hydrogenation, single bond dehydrogenation, steroid alcohol
oxidation (e.g., 17β-OH → 17C=O), steroid ketone reduction (e.g., 17C=O → 17β-OH), and double bond isomerization can be considered as other potential transformation mechanisms of these hormones by bacteria. Nocardia and Arthrobacter are responsible for degradation of progesterone in soil by cleavage of the B-ring, beginning with the introduction of a double bond between carbons 1 and 2, and hydroxylation at carbon 9, which causes aromatization of the A-ring, cleavage of the B-ring, and the formation of a labile metabolite. (Donova, 2007; Young and Borch, 2009). The possible hydroxylation, steroid ketone reduction, double bond hydrogenation, and single bond dehydrogenation of these hormones by microalgae and fungi is also reported by Faramarzi et al. (2008). The moisture content, redox conditions and temperature demonstrated an influential effect on microbial activity and as a result, the degradation of steroid sex hormones (Xuan et al., 2008). On the other hand, the coexistence of other organic pollutants such as veterinary antibiotics can significantly influence the biodegradation of hormones by affecting the microorganisms (Xuan et al., 2008).

7.7 Conclusion

The biotic degradation and abiotic transformation of three natural female sex hormones and the influence of slow pyrolysis biochar as a soil amendment on these mechanisms were investigated through a two stage, laboratory scale experiment under simulated field conditions. The hormonal content of soil samples spiked with a similar concentration of each hormone under four different treatments, including sterile and non-sterile soil, and soil-biochar were analyzed after 48 and 120 hours to determine the degradation kinetics of each hormone under photo-degradation, biodegradation or chemical transformation, using a first-order degradation model. The comparison of analyzed sterile soil samples exposed to simulated sunlight radiation and covered soil samples demonstrated the statistically insignificant influence of photo-degradation on the dissipation of all three hormones. Less than 10% of E2 and P were transformed by photo-degradation. Even though E1 demonstrated a higher potential for photo-degradation than the other two hormones, the effect of photo degradation for E1 was not statistically significant. The presence of biochar in soil media did not demonstrate a significant influence on the photo-degradation of all three hormones. The biodegradation and chemical
transformation mechanisms demonstrated a critical effect on the persistence and dissipation of these hormones; however, no significant difference was observed on the degradation behavior of hormones in the presence of biochar as compared to soil samples without biochar. E2 was dominantly degraded through biodegradation; however, progesterone and E1 were mainly decomposed by chemical transformation. The half-life of all three hormones was less than 3 days in non-sterile soil samples whereas in the sterile media, they were more persistent, which possibly may be due to the reaction conditions under which these chemical transformations take place.
Chapter 8
Summary and Conclusions

The main objective of this study was to develop an economically-feasible remediation technique to reduce the environmental and biological consequences of hormonal pollution caused by manure application in agricultural fields. Both laboratory and field-lysimeter studies were conducted to meet the objective. The first study was designed to assess the sorption ability and desorption resistance of two types of biochars (fast and slow pyrolysis biochar) in a sandy soil, contaminated by three major manure-borne steroidal sex hormones (17β-estradiol, estrone and progesterone) through a set of batch equilibrium experiments.

The preliminary conclusions, drawn from this laboratory study can be summarized as follows:

1. Soil-biochar amendment can sorb the three female sex hormones in a significant way.
2. Once sorbed, soil-biochar amendment was found to retain the hormones and thus could play a significant role in reducing the hormonal pollution.
3. The sorption and desorption isotherms indicated that, in both sorption and desorption batch equilibrium experiments, slow pyrolysis biochar showed a better support for our hypothesis of the remediation capacity of biochar to reduce hormonal pollution in agricultural soil, amended with animal manure.

The second study was conducted at an outdoor field-lysimeters site and it evaluated the retention ability of slow-pyrolysis biochar as a soil amendment to reduce the manure-borne hormonal pollution. Both poultry and swine manure were used in this study.

The following conclusions are drawn from this study:

1. The statistical analysis of spatial-temporal stratification of hormones in lysimeters under both soil and soil-biochar treatments demonstrated the significant effect of biochar for reducing the manure-borne hormonal pollution in the soil and water matrix.
2. Significantly lower loads of all three hormones were measured at different soil depths and in the lysimeter leachate over time in the lysimeters amended with 1% slow pyrolysis biochar.

As the final step of this research, the biotic degradation and abiotic transformation of three natural female sex hormones and the influence of slow pyrolysis biochar as a soil amendment on these mechanisms were investigated.

1. Biodegradation and chemical transformation mechanisms were found to be the dominant mechanisms affecting the persistence and dissipation of female sex hormones in the soil medium.

2. No significant difference was observed in the degradation behavior of the three hormones in biochar-amended soil.

8.1 Recommendation for further studies

Biochar can be proposed as a multi-purpose organic soil amendment with remediation potential to reduce surface water hormonal contamination and to eliminate the detrimental biological and adverse health effects of animal manure, while improving soil quality.

The following areas should be explored further in future studies:

1. In this study, the retention ability of biochar was limited to only one type of biochar and one type of soil. It is recommended that similar experiments may be conducted in other soil types, for example, sandy loam and loamy soils using different type of biochars, obtained from different feedstocks.

2. The competitive sorption ability and desorption resistance of biochar should be evaluated in presence with other micro pollutants, such as antibiotics and nanoparticles.

3. Another consideration is the potential blockage of the biochar’s pores by the adsorbed soil organic matters, leading to a decrease in its sorption capacity. Consequently, the interaction of biochar with other soil components should also be investigated.
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